EVALUATION OF NUTRITIONAL STRATEGIES FOCUSED ON EXOGENOUS ENZYMES AND SYNTHETIC AMINO ACIDS AIMED AT MAXIMIZING BROILER PERFORMANCE AND NUTRIENT UTILIZATION

A Dissertation

by

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ABSTRACT

The objective of this research was to evaluate nutritional strategies focused on exogenous enzymes and synthetic amino acids aimed at maximizing broiler performance and nutrient utilization. Four experiments were conducted in total. Three experiments evaluatedthe use of exogenous enzymes beta-mannanase and xylanase, and a fourth experiment determined the digestible sulfur containing amino acid requirement. In Experiment 1, the dietary inclusion of beta-mannanase improved the growth performance of broilers fed a low energy diet to that of birds fed a high energy control diet. Growth improvement was related to increases in ileal digestible energy (IDE) during the early rearing period (increased by 55 kcal/kg on d17). This observation is linked to the presence of a higher level of soybean meal in the earlier phases of rearing which contains galactomannan, the substrate for beta-mannanase.

Experiment 2 further investigated the impact of beta-mannanase in diets with varying galactomannan concentration. Galactomannan content was increased with the addition of guar gum which is a highly concentrated galactomannan. Increasing levels of galactomannan in diets without beta-mannanase negatively influenced growth performance by increasing intestinal viscosity and decreasing IDE. Improvements in digestibility and performance associated with beta-mannanase inclusion were related to the concentration of galactomannan in the diet.

In Experiment 3, the necessity of synthetic amino acids was demonstrated as all performance parameters evaluated showed strong relationships for digestible sulfur

amino acid (dSAA) level, except for feed intake. The dSAA requirements that maximize d 49 body weight and body weight gain and minimized mortality adjusted FCR and cumulative FCR were 0.793, 0.800, 0.764, and 0.772%, respectively.

During experiment 4, the impact of xylanase on multiple corn sources was evaluated. Xylanase increased starter feed consumption and d 18 body weight. Corn source nutrient variation resulted in growth performance differences, and xylanase impact was greater in corn sources that resulted in poorer performance. In summary, this demonstrates the performance benefits of exogenous enzymes and synthetic amino acids which the poultry industry can use to reduce production costs.

DEDICATION

This dissertation is dedicated to those who have supported me throughout the years from developing interest and knowledge of agriculture and laying down the foundation for the drive to be successful.

To my grandparents, thank you for the love and support that you have provided to me throughout my life. You have always been there to encourage and support me in every encounter in life.

To my mom and brother, thank you for your unwavering love and support. You have always been there for me through the highs and lows and always found a way to provide anything that I ever needed. I can't thank you enough for the support you have given over the years.

To my ag teachers, thank you both for the opportunity that you provided me with to go through such an amazing program. The knowledge, skills, and qualities I gained from your guidance are the main reason for the success I have achieved. I wouldn't be in the position I'm in today if it wasn't for you both. Thank you for always being there and for the opportunities you have helped provide me with.

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Finally to my wife, words can't begin to describe how much you have meant to me throughout this entire process. You have been there everyday offering love and support even though I never made it easy for you and for this I'm forever grateful. It has been a long road and I'm lucky to have had you by my side throughout the journey. You've been my rock and I couldn't have made it without you. Thank you so much for everything along the way.

NOMENCLATURE

AA	Amino Acids
AME	Apparent Metabolizable Energy
AOAC	Association of Official Agriculture Chemists
BMY	Breast Meat Yield
BV	Bioavailability
BW	Body Weight
BWG	Body Weight Gain
cFC	Cumulative Feed Consumption
cFCR	Cumulative Feed Consumption Ratio
cP	Centipoise
d	Day
dAA	Digestible Amino Acid
DDGS	Distillers' Dried Grains with Solubles
DFM	Direct-Fed Microbials
dLys	Digestible Lysine
DLM	DL-Methionine
dMet	Digestible Methionine
dSAA	Digestible Sulfur Amino Acids
FC	Feed Consumption
FCR	Feed Consumption Ratio

GLM	General Linear Model
GM	Galactomannan
HMTBa	DL-2-hydroxy-4-methylthio butanoic acid
IACUC	Institutional Animal Care and Use Committee
IDE	Ileal Digestible Energy
IgM	Immunoglobulin M
L-Lys	L-Lysine
L-Met	L-Methionine
MBM	Meat and Bone Meal
MCT_1	Monocarboxylate Transporter 1
Met	Methionine
NC	Negative Control
NIR	Near Infrared Reflectance
NRC	National Research Council
NSP	Non-Starch Polysaccharides
PC	Positive Control
point	One point is equal to 0.01 FCR
ppm	Parts per Million
PSI	Protein Solubility Index
QB	Quadratic Broken-Line
SAA	Sulfur Amino Acid
SBM	Soybean Meal

tLys	Total Lysine

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CHAPTER I

INTRODUCTION

Feed production is the largest cost for broiler producing companies. The ability to implement strategies to reduce this cost is an important consideration within the poultry industry. Multiple methods are available for nutritionists to utilize in reducing diet costs while maintaining or enhancing current broiler performance. Examples include inclusion of direct-fed microbials (DFM), exogenous enzymes, AA, and alternative ingredients. Exogenous enzymes, including β-mannanase and xylanase, have been implemented in broiler diets for several years, and numerous publications are available describing their benefits. Cowieson and colleagues (2010) reported that the commercialization of exogenous enzymes is estimated to save global animal agriculture between $3 and 5 billion dollars annually. It is important to continue to evaluate multiple sources of β-mannanase and xylanase enzymes to continually improve performance and digestibility, as well as adapt to changes in demands of the broiler as genetics change over time.

Inclusion of synthetic amino acid (AA) to meet maintenance and growth requirements of broilers is a beneficial tool for poultry nutritionists and has been extensively researched in young broilers. Amino acid concentration is of greater concern in young broilers while energy becomes the primary focus as broilers age. Diets are currently formulated on an ideal AA basis with the most limiting AA being of main concern. The most limiting AA are dependent on the type of diet fed to broilers. In the case of corn-soybean diets, the first limiting AA is methionine. Sulfur amino acids such

as methionine are essential for growth, feather formation, and methyl donation (Garcia and Batal, 2005). The ratio of methionine to lysine included into broiler diets has changed over the last several years from the National Research Council (NRC, 1994) sulfur amino acid (SAA) ratio at 84% to an increase of 4% in SAA ratio (88%) in Lesson and Summers (2005). Recent research trials (Goulart et al., 2011; Rostagno et al., 2011) are now suggesting a SAA range of 75 to 78% which is much lower than the previous years. The methionine requirement of young broilers is well documented. Current research availability focusing on AA requirements for the modern broiler is limited and is needed for more accurate diet formulation.

Ingredient variation is a major concern of broiler nutritionists for accurate diet formulation. Knowing the true nutritive value of ingredients being used would allow for improved utilization of nutrients and potentially reduce diet costs. There are numerous factors that can effect ingredient variation such as genetics, physical composition, anti-nutritive factors, agronomic conditions, post-harvest processing, and storage conditions (Rooney and Pflugfelder, 1986; Cowieson, 2005; Gehring et al., 2012; Gayral et al., 2015). Cowieson (2005) reported that differences in corn samples can yield variability in apparent metabolizable energy (AME) of more than 400 kcal/kg. A difference in AME of this magnitude could greatly impact broiler performance. Therefore, having accurate ingredient information is imperative for efficient diet formulation. One way this can be done is through technology such as Near Infrared Reflectance (NIR) spectroscopy. The NIR provides rapid results allowing for adjustments prior to diet formulation. Several calibrations or histories of ingredient profiles are available for nutrient prediction.

The objective of this research was to evaluate multiple strategies including exogenous enzyme inclusion, determination of methionine requirement, and ingredient variability to improve broiler performance and increase nutrient absorption.

CHAPTER II

LITERATURE REVIEW

Improvement of Nutrient Utilization through the Inclusion of Exogenous Enzymes

Non-Starch Polysaccharides. Numerous grains and grain products are available for poultry producers to utilize in diet manufacturing. Selection of ingredients for diet formulation relates to what is most readily available at the best cost (Bedford, 1995). Corn and soybean meal are common ingredients used in U.S. poultry diets and contain various amounts of fibrous material classified as non-starch polysaccharides (NSP). Some of the common NSP of concern in corn and soybean meal include arabinoxylans, β-glucans, pentosans, arabinogalactans, mannans, galactomannans, xylans, oligosaccharides, cellulose, hemicellulose, and pectins (Bacic et al., 1988; Choct, 2006; Caprita et al., 2010; Slominski, 2011) (Figure 2-1).

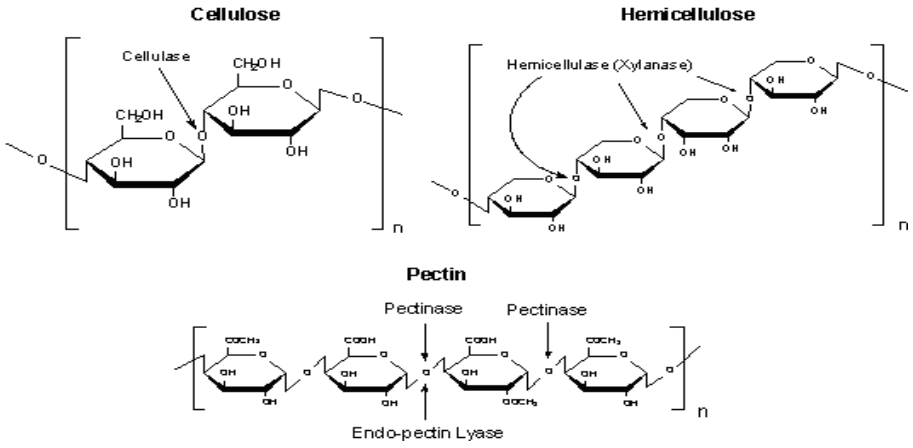


Figure 2-1. Structural models of cellulose, hemicellulose, and pectin with proposed cites of hydrolization (Carpita and McCann, 2000).

Corn, the main source of energy in poultry diets with an AME value of 3344 kcal/kg (Cowieson, 2005), contains negligible amounts of soluble NSP and approximately 8% insoluble NSP predominantly consisting of arabinoxylans and β-glucans (Choct, 2006; Slominski, 2011). Corn is also rich in starch content, containing more than 600g/kg which contributes approximately 60% of the AME content of poultry feeds (Weurding et al., 2001a, 2001b). A challenge with starch digestion in corn is due to the starch being imbedded in the protein matrix. This leads to increased protein-starch interactions (Rooney et al., 1986; Cowieson, 2005; Gayral et al., 2015). Many factors such as plant species, inhibitors, structure of the starch/lipid/protein matrices, and anti-nutritive factors can impact corn starch digestibility (Leigh, 1994; Brown, 1996; Collins et al., 1998; Cromwell et al., 1999; Collins and Moran, 2001; Gehring et al., 2012). The two molecules comprising starch are amylose and amylopectin, which are polymers of D-glucose (Carre, 2004; Tester et al., 2004; Cowieson, 2005). Starch digestion should be defined as the complete hydrolysis into glucose monomers (Cowieson, 2005). However, not all consumed starch is digested directly by the animal (Svihus, 2001). Digestibility of starch is challenging to measure precisely. Previous authors have usually determined it as the disappearance of starch rather than the digestibility of starch (Cowieson, 2005).

Soybean meal (SBM), which is the most widely used source of vegetable protein in the U.S., contains 3% soluble NSP and 16% insoluble NSP (Irish and Balnave, 1993). The soluble and insoluble NSP consists predominantly of mannas (Slominski, 2011). Mannans can occur in multiple forms such as glucomannans, galactomannans,

glucogalactomannans, and glucuronomannans in plant cell walls (Aman and Graham, 1990). Soluble β-galactomannan is a polysaccharide composed of D-mannose units attached by β-1, 4 linkages, with galactose or glucose often found attached to the β-mannan backbone (Carpita and McCann, 2000) (Figure 2-2).

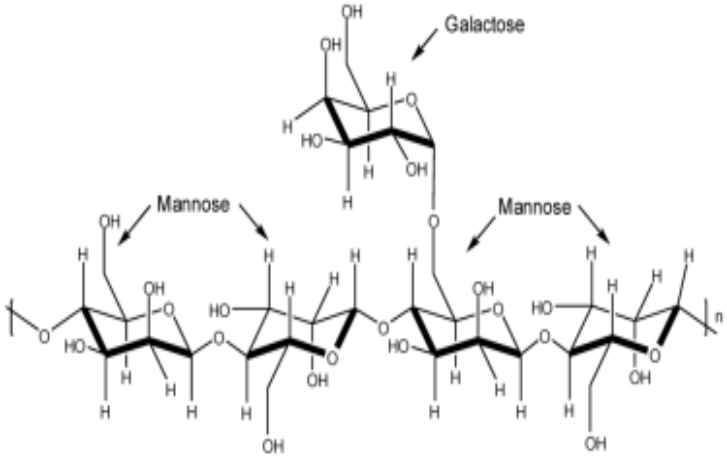


Figure 2-2. Segment of galactomannan showing mannose backbone with a branching galactose unit (Teixeira et al., 2014).

Beta-mannans have been characterized as an immunogenic substance due to its ability to illicit an immune response (Jackson et al., 2003, 2004; Klasing, K., 2007; Dale et al., 2008). These immunogenic factors are not deleterious in their own right, but cause an unnecessary immune response (Jackson et al., 2003, 2004; Dale et al., 2008). Acute phase proteins are excellent indicators of innate immune system activity. Therefore monitoring the levels of these proteins is an effective method for determining the innate immune response. Dale and co-workers (2008) demonstrated a reduction in levels of acute phase proteins in the plasma of birds when diets were supplemented with β-mannanase. An immune response in the presence of a pathogen is necessary, but in

the absence of a pathogen is counter-productive. Klasing (2007) has described the deleterious effects of the immune response on productivity in detail indicating that an acute phase response may divert up to 10% of ingested nutrients away from productive processes.

Viscosity. Viscosity is a physiochemical property of nutritional importance regarding NSP along with hydration properties, cation exchange capacity, and organic compound absorptive properties (Bach Knudsen, 2001). Viscosity severity is directly related to the molecular weight and size of the structure, ion charged groups, surrounding structures, and NSP concentration (Smits and Annison, 1996). Viscosity is a function of the degree of polymerization of the carbohydrate (Izydorczyk and Biliaderis, 1992; Nilsson et al., 2000), as well as the concentration of soluble carbohydrate (Cowieson et al., 2005). Choct and colleagues (2010) suggested that NSP influence on increased digesta viscosity may lead to increased water consumption, potentially resulting in an increase in fecal moisture and wet litter incidence. Mongastric animals such as poultry lack the digestive enzymes to breakdown dietary NSP (Bedford, 1995; Meng et al., 2005), leading to an increase in intestinal viscosity. Increased intestinal viscosity can lead to a reduced rate of digesta passage within the bird's small intestine (Annison, 1993; Bedford, 1995; Bedford and Schulze, 1998; Choct et al., 2010). Other anti-nutritive effects of increased intestinal viscosity are decreases in nutrient uptake and enzyme contact, increase in intestinal mass and increase the rate of mucosa cell turnover (Gee et al., 1996; Danicke et al., 2000; Teirlynck et al., 2009). These anti-nutritive effects ultimately reduce exposure to brushy border enzymes

impeding digestion, and limiting absorption of nutrients through enterocytes (Bedford, 1995; Gee et al., 1996). Research has shown that viscous feeds have had greater impacts on younger birds when compared to older birds with more developed digestive tracts (Yasar and Forbes, 2000; Lee et al., 2003 a, b; Lee et al., 2005).

Nutrient Encapsulation. Entrapment of nutrients by polysaccharides in cereal grain cell walls has been suggested to inhibit access of digestive enzymes to starch, protein, and fat (Annison, 1993; Meng, 2005). Further, NSP can be divided into water-soluble or water-insoluble fractions (Annison, 1993). Monogastric animals such as poultry lack the digestive enzymes to hydrolyze the cell wall and increase nutrient availability to the bird. It has been reported that water-insoluble NSP are indigestible by poultry with water-soluble NSP representing a potential source of digestible nutrients (Carre, 1993). However, water-soluble NSP possess anti-nutritive properties through the physical encapsulation of nutrients in combination with reducing nutrient digestibility through increased viscosity (Annison, 1993; Bedford, 1995; Choct et al., 2010). It has been suggested that the reduction in nutrient digestibility decreases the AME of the diet and increases FCR (Annison, 1993). With exogenous enzyme inclusion in reduced energy diets, increases in ileal digestible energy (IDE) between 90-112 kcal/kg have been observed (Cowieson and Adeola, 2005; Cowieson et al., 2006; West et al., 2007; Masey O'Neill et al., 2012; Williams et al., 2014; Latham et al., 2016). West and co-workers., (2007) estimated a 1 to 2% increase in AA digestibility with enzyme inclusion while Latham and colleagues., (2016) reported an average of 2.7% increase in total AA digestibility.

Inclusion of exogenous enzymes in poultry diets to improve nutrient utilization is a common practice in the poultry industry. There are numerous publications available which describe the effects of enzyme inclusion. Research has shown the inclusion of exogenous enzymes in feeds to be advantageous for improving broiler performance parameters through increasing nutrient digestibility, decreasing intestinal viscosity, and ultimately reducing feed costs (Bedford and Classen, 1992; Bedford and Morgan, 1996; Lazaro et al., 2003; Meng and Slominski, 2005; Choct, 2006). Enzymes have been introduced to improve performance by hydrolyzing indigestible bonds in the plant's cell walls into smaller fragments allowing for increased digestibility and improved bird performance (Coppedge et. al., 2012). Bedford (2000) reported that enzymes are supplemented in poultry diets to increase the feeding value of ingredients, reduce the variation in nutrient quality of ingredients, and reduce the incidence of wet litter. However, the level of effectiveness of enzyme inclusion depends on several factors such as ingredients and amount of substrate available. Enzyme supplementation has been more effective in high fiber based diets (Bedford and Classen, 1992; Bedford and Morgan, 1996; Choct, 2006) compared to corn and soy based diets which have produced variable results (Bedford and Classen, 1992; Meng and Slominski, 2005; Choct, 2006). Enzymes have different mechanisms of action depending on the enzyme. Energy releasing enzymes disrupt the cell wall structure and release encapsulated nutrients, verses energy sparing enzymes which breakdown NSP that could elicit an immune response, thereby preventing unnecessary energy expenditure.

Xylanase Inclusion. Carbohydrases such as xylanase, when included in broiler diets, have been shown to increase nutrient availability and significantly improve broiler performance in reduced energy diets (Bedford and Classen, 1993; Wang et al., 2005; Esmaeilipour et al., 2011; Coppedge et al., 2012; Masey O'Neill, 2012; Williams et al., 2014). Xylanase (Figure 2-3) inclusion is a common practice in the broiler industry to provide an energy releasing effect by hydrolyzing plant cell walls and liberating previously unattainable nutrients for the bird. The ability of xylanase to improve broiler performance in reduced energy diets may reduce the overall feed costs for the producer.

The two primary modes of action for the NSP enzymes are reducing the viscosity of feed stuffs and improving nutrient digestibility (Cowieson et al., 2006), or the release of previously encapsulated nutrients termed the 'Cage Effect' theory (Bedford, 2000). Regardless of the mode of action, inclusion of carbohydrase results in an increased rate of nutrient digestibility (Bedford, 2000). This is important to understand because it moves the site of digestion and absorption of starch and protein to a more anterior part of the digestive tract where the bird has a competitive edge over the resident microflora (Bedford, 2000). It has been stated that supplementing carbohydrases in corn-soy diets enhanced NSP, protein and energy utilization (Marsman et al., 1997; Douglas et al., 2000; Kocher et al., 2002; Meng et al., 2005), however failed to result in better growth performance of poultry.

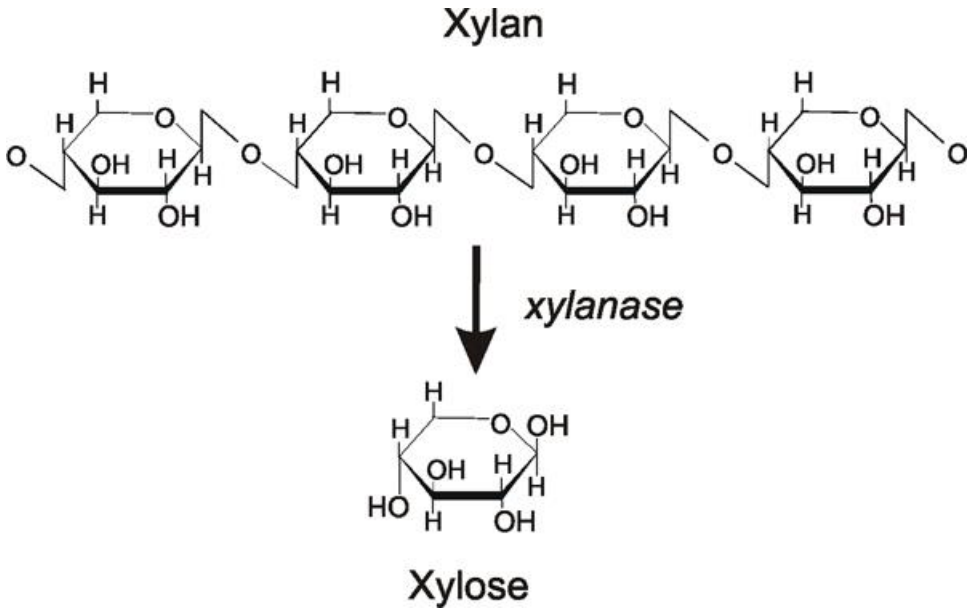


Figure 2-3. Xylanase structural model (Vaijayanthi et al., 2016).

Beta-Mannanase Inclusion. The exogenous enzyme β-mannanase targets β-galactomannan in which the main source is SBM. Beta-galactomannans are then converted into mannose oligosaccharides through enzymatic degradation. Studies have shown that inclusion of β-mannanase in poultry feed have increased AME and BWG, and decreased FCR (Daskiran et al., 2004; Jackson et al., 2004; Lee et al., 2005; Zangiabadi and Torki, 2010). Inclusion of β-mannanase has also been shown to have immunological benefits by reducing lesion development in broilers subjected to a necrotic enteritis challenge model through a combined *Eimeria* species and *Clostridium perfringens* challenge (Jackson et al., 2003). In addition, Williams and colleagues (2014) reported that including β-mannanase significantly improved broiler performance in a reduced energy diet. Jackson and co-workers., (2003, 2004) suggested that

improvements may be due to the immunological benefits of β-mannanase. The mechanism of immunological benefits associated with β-mannanase inclusion is unclear. Mannans are components of the surface of multiple types of pathogens including fungus, bacteria, and viruses (Hsiao et al., 2006). Jackson and co-workers (2004) demonstrated that β-mannan can stimulate the innate immune response, potentially leading to unnecessary energy expenditure (Hsiao et al., 2006). This feed induced immune response leads to increasing proliferation of macrophages and monocytes, resulting in increased cytokine production and increase in immunoglobulin M (IgM) concentrations (Jackson et al., 2004; Hsiao et al., 2006; Zou et al., 2006). Jackson and colleagues (2004) observed that reducing the amount of β-mannan content within the intestine decreased the energy expenditure through an active innate immune response, ultimately leading to more efficient nutrient utilization and energy expenditure.

Potential Nutrient Source. Corn-SBM based feeds are relatively low in NSP concentration, but an estimated 400 to 450 kcal/kg in broiler diets can remain undigested (Cowieson, 2010). The inclusion of exogenous enzymes to potentially increase nutrient availability of these ingredients is a viable option. As previously described, inclusion of carbohydrases can ameliorate the negative effects of the dietary NSP through the hydrolization of the cell wall, creating more opportunity for digestion and absorption.

Starch is one of the most important polysaccharides with 90 to 95% digestibility occurring in the chicken small intestine through endogenous enzyme activity (Annison, 1993). Xylanase supplementation in corn-SBM diets could provide benefits to energy digestion through the breakdown of NSP, even with NSP content at low concentrations

(Slominski, 2011). Masey O'Neill and colleagues (2012) suggested that xylanase can be assigned an energy value since it contributes to the overall energy level. The reduction in energy is accomplished by reducing the amount of supplemental fat in the diet, which is typically an expensive ingredient, and replacing it with corn which is typically less expensive than fat (Masey O'Neill et al., 2012). This has become a common practice in modern feed formulation.

It is well documented that reducing the energy in broiler diets will result in negative impacts to broiler performance and ileal digestibility of nutrients (Cowieson et al., 2010; Coppedge et al., 2012; Masey O'Neill et al., 2012; Singh et al., 2012; Williams et al., 2014). Coppedge and colleagues (2012) reported when energy was reduced by 133 kcal/kg, there was a decrease in BW through d 26 when compared to the positive control (PC) diet with no reduction in energy. Masey O'Neill and colleagues (2012) observed a significant increase in FCR through d 42 when reducing dietary energy. In a study conducted by Williams and colleagues (2014), diets that were reduced in energy by 88 kcal/kg in the starter and grower and 132 kcal/kg for the remaining phases compared to the control, a significant reduction in BW and increase in FCR was observed when compared to the PC. However, the inclusion of exogenous carbohydrases can overcome the negative impacts of energy reduction resulting in reduction in diet cost. Coweison and colleagues. (2010) reported that xylanase inclusion in a 110 kcal/kg reduced energy diet improved FCR by 5 points to levels similar to broilers fed the standard energy diet. Also observed was an increase in IDE during the finisher phase by 91 kcal/kg when compared to un-supplemented diets. With the energy

reductions previously mentioned, Williams and colleagues, (2014) reported significant improvements in BW and FCR with xylanase and/or β-mannanase supplementation. Supplementing carbohydrases in corn-soy diets enhanced NSP, protein and energy utilization in poultry (Marsman et al., 1997; Douglas et al., 2000; Kocher et al., 2002). Increases in IDE of >100 kcal/kg and a 2.7% increase in AA digestibility have been observed (Cowieson and Adeola, 2005; Latham et al., 2016).

Amino Acid Utilization in Broiler Feed

Within the poultry industry, the use of digestible amino acid values and ratios are comprehensively used in diet formulation. Diets are currently formulated on an ideal AA basis with the most limiting AA being of primary concern. The limiting AA will be dependent on the type of diet being fed to broilers. Typically for standard corn-SBM diets, the most limiting AA will be methionine or SAA, lysine, threonine, and valine (Kidd et al., 2004; Corzo et al., 2007; Dozier III et al., 2008; Zhang and Guo, 2008; Mejia et al., 2012). Determining the minimums for essential digestible amino acids (dAA)is dependent on digestible lysine (dLys) level as they are defined as ratios relative to lysine. This method is widely used and simplifies diet formulation for nutritionist by allowing for adjustment in total AA density by altering the dLys level. Increased broiler growth rates in recent years, attributed to selection and nutrition, have resulted in major advances in broiler production and changes in amino acid requirements. Recommendations in which AA ratios are included in broiler diets have varied over the last several years from the NRC (1994) with a SAA ratio of 84% to Lesson and Summers (2005) with a SAA ratio of 88% to lysine. More recent research trials (Goulart

et al., 2011; Rostagno et al., 2011) have recommended SAA levels at a much lower rate ranging from 75 to 78%. Genetically, the modern broiler is constantly changing for improvements in growth rate, FCR, meat yield, and livability. Therefore, the nutritional requirements may also be changing to maximize the genetic potential of the animal.

Synthetic Amino Acids. To assist in balancing and meeting the dAA requirements of broilers, numerous synthetic AA are currently being used in diet formulation. These synthetic AA are available in multiple forms such as powder or liquid and have numerous suppliers and manufacturers available. The use of synthetic AA is an important tool for nutritionist allowing for least cost formulation while also ensuring poultry are receiving the dietary AA levels required for growth and maintenance, ultimately achieving maximum profitability. The majority of synthetic AA such as L-Lys/L-Lys SO_4/L-Lys HCl, L-Threonine, L-Tryptophan, L-Methionine, L-Arginine, and L-Valine are manufactured through fermentation while DL-Met and DL-2-hydroxy-4-methylthio butanoic acid (HMTBa) are chemically manufactured (Zou et al., 2015).

Digestible Lysine Requirements. As previously mentioned, minimum essential AA ratios are determined depending on dLys levels. This method is of major importance economically because it determines the amount of total AA inclusion in broiler diets. The dLys levels will be dependent on broiler age and feeding phase throughout the grow-out process. Sriperm (2011) conducted a titration study for dLys level for the grower d 15 to 35, early finisher d 35 to 42, and late finisher d 42 to 49 phases. Observations for the grower phase resulted in different requirements for dLys

depending on the performance parameter evaluated. The dLys level which maximized BWG, FCR, and breast meat yield (BMY) were 1.126, 1.388, and 1.135%, respectively. For d 35 to 42, observations showed that maximum BWG and BMY were achieved at a dLys level of 0.996 and 0.920%, respectively. For d 42 to 49, observations showed that maximum BWG and BMY were achieved at a dLys level of 0.992 and 1.004%, respectively. It was suggested that the dLys level that maximized performance and processing parameters is dependent upon the length of the growth phase.

Manufacturing Process of DL-Methionine and DL-2-Hydroxy-4-Methylthio Butanoic Acid. Two common supplemental methionine sources used in commercial poultry diets are DL-methionine (DLM) and HMTBa. The DLM product is an AA in the form of a powder and has a purity and bioavailability of 99 and 100%, respectively. It is absorbed at the intestinal level by bo+ and Bo+ transport proteins (Richards et al., 2005). The HMTBa is an acidic analog that is converted into an AA within the body. It is marketed in liquid form and has a purity and bioavailability of 88 and 100%, respectively. It is passively absorbed along the gastrointestinal tract and actively by the MCT_1 transport protein within the small intestine (Richards et al., 2005). Both methionine sources are chemically synthesized in the D and L form, and absorbed and converted to L-Met in the body. Both DLM and HMTBa are constructed chemically from mineral and organic derived compounds (Figure 2-4).

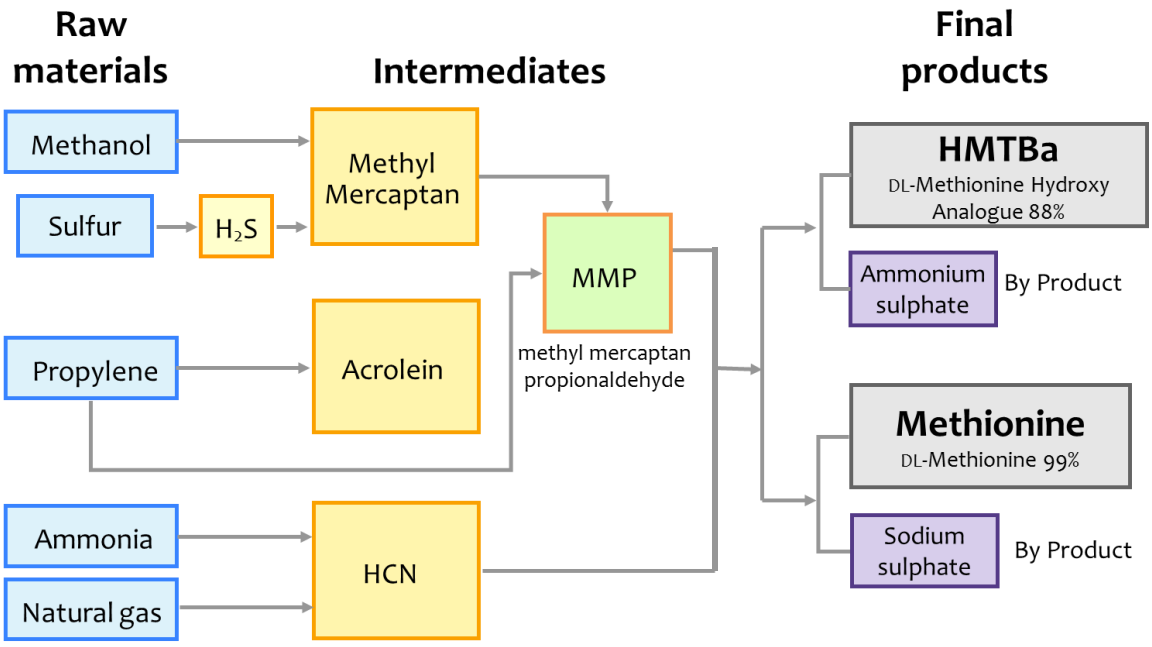
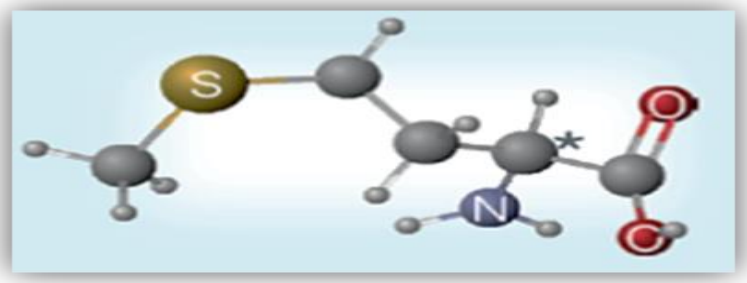


Figure 2-4. Manufacturing process of DL-Methionine and DL-2-Hydroxy-4-Methylthio Butanoic Acid (The complex process of manufacturing methionine. AllAboutFeed.net 2011).

The chemical difference between the two methionine sources is the carboxyl group which is attached to the DLM molecule and the hydroxyl group which is attached to the HMTBa molecule (Dibner, 2003) (Figure 2-5 a, b).

(a)



(b)

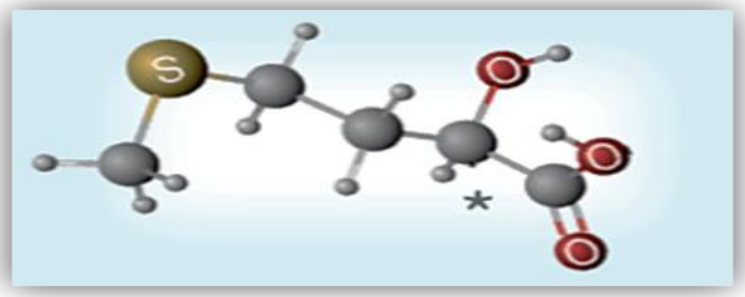


Figure 2-5. (a) DLM molecule and (b) HMTBa molecule (Dibner, 2003).

Digestible Methionine or Sulfur Amino Acid Requirement. Methionine (Met) is classified as an essential AA because it cannot be synthesize by mammals or poultry and must be ingested through diet. Methionine content is relatively low in plant material, therefore supplementation with synthetic Met is required to ensure that requirements are met. Methionine is the most limiting AA in standard corn-SBM broiler diets. Sulfur AA are essential for growth, feather formation, and methyl donation (Garcia and Batal, 2005). As previously stated, SAA recommended requirements have changed over the last several years. Current AA requirements for the modern broiler are limited and in need of revision for more accurate diet formulation. In previous research investigating the Met requirement and dSAA:dLys ratio, Zhang and Guo (2008) reported

a linear decrease in FCR as HMTBa supplementation increased in broilers from 21 to 42 days suggesting that increased levels of HMTBa inclusion improves FCR. Goulart and colleagues (2011) observed that broilers from 36 to 42 days-of-age reached maximum performance at a dSAA:dLys ratio of 72%, although the requirement to maximize breast weight was achieved at dSAA:dLys ratio of 76% according to Dozier III and colleagues (2013). Reports by Carew, Mcmurtry, and Alster (2003) and Attia , Hassan, Shehatta, and El-Hady (2005) suggest that broilers increase consumption in order to accommodate for a Met deficiency. Other researches (Kiraz and Sengul, 2005; Bunchasak, 2009) have reported data which contradicts these findings suggesting that a Met deficiency may reduce appetite. More recent publications by Zhang and Guo (2008) and Goulart et al. (2011) reported that feed consumption (FC) was not influenced by Met supplementation.

Methionine requirement can be influenced by many factors including rearing conditions, sex, genetics, crude protein levels, and addition of drugs or other feed ingredients (Petel et al., 1980; Jensen et al., 1989; Garcia and Batal, 2005; Dilger et al., 2007; Lumpkins et al., 2007; Karimi et al., 2011). Copper is commonly included in current broiler diets because of its antibacterial or bacteriostatic properties to serve as an alternative for antibiotic growth promoters (AGPs) and improve broiler performance (Ewing et al., 1998; Pesti and Bakalli, 1998; Arias and Koutsos, 2006; Karimi et al., 2011). Although studies have shown copper inclusion to improve broiler performance parameters, others have reported that copper inclusion at higher levels (>250 ppm) have had variable results (Jensen et al., 1989; Banks et al., 2004; Arias and Koutsos, 2006; Pang and Applegate, 2007) and even deleterious effects on performance (Persia et al.,

2004; Luo et al., 2005). Jensen and co-workers (1989) reported inconsistent results over two experiments with one resulting in a significant interaction between copper and Met, with larger improvements in feed efficiency with Met supplementation in the presence of copper. However, observations for the second trial resulted in greater improvements in BWG in males in the absence of copper. With inconsistent reports of copper inclusion effects on Met, a conclusion cannot be reached on copper's influence to improve or degrade the benefits from Met.

DLM vs. HMTBa Supplementation. When comparing DLM and HMTBa Met sources on broiler performance results have been variable. Previous research has concluded that both Met sources can be absorbed at equivalent rates (Knight and Dibner, 1984) and that the compounds are considered to be equal on an equimolar basis (Daenner and Bessei, 2003; Motl et al., 2005; Zou et al., 2015). Dibner (2003) stated that HMTBa may be more highly absorbed because more tissues are capable of absorption when compared to DLM. Other researchers have reported obtaining maximum performance when using DLM as compared to HMTBa (Vaxquez-Anon et al., 2006; Sauer et al., 2008; Vedenov and Pesti, 2010). Vazquez-Anon et al. (2006) stated that HMTBa was able to outperform DLM at high concentrations of dSAA when comparing gain-response curves to dietary concentrations of the Met sources. However, at lower dSAA concentrations, broilers fed DLM resulted in greater BWG when compared to broilers fed HMTBa. Zou et al. (2015) suggested that HMTBa can outperform DLM by decreasing the amount of feed necessary when supplementing Met at higher levels.

Ingredient Variation in Diet Formulation

The market for quality ingredients for poultry feed formulation includes a large number of cereal grains and their by-products. There is a continued pressure on the demand of feed ingredients due to the many uses of these ingredients such as ethanol production, drought in recent years, and ingredient variability. When demand increases for these ingredients, it has led to an increase in ingredient costs, thus forcing nutritionists to find ways to maximize the nutrient utilization of feed stuffs in order to reduce feed costs. Many times in diet formulation, ingredients are considered to be equal in nutritive value, even when ingredients are received from different geographical locations. This can lead to inaccurate diet formulation due to ingredient variability. Resources such as the Nutrient Requirements of Poultry (NRC, 1994) are commonly used for guidance. Not only are these ingredients assigned a single value, regardless of geographical location, genetics, and post-harvest conditions, the values are over 20 years old, and thus may not reflect ingredients available in the market currently. Technological advances have been made in the poultry industry to aid in developing more accurate or consistent methods of determining the nutritional value of ingredients.

Alternative Ingredients. Ethanol (a biofuel largely produced by corn) utilization has exponentially increased in recent years, thus resulting in an increased demand for corn (Campasino et al., 2015). This has put pressure on corn prices and led to an increase in cost for livestock producers. In 2002, 11% of the United States corn crop was being used for ethanol production (Donohue and Cunningham, 2009). Corn utilization for ethanol production was increased to 30% in 2008, which directly

influenced feed costs for poultry producers (Donohue and Cunningham, 2009). Between the years of 2006 and 2008, it was estimated that feed costs in the poultry industry increased as much as $9.36 billion dollars in the United States alone (Donohue and Cunningham, 2009). The cost increase caused nutritionists to use less desirable ingredients at higher levels in order to reduce production costs. The use of by-products such as distillers' dried grains with solubles (DDGS), which is a by-product of ethanol production, is commonly incorporated in feeds due to its high availability and relatively low costs. Although variable, high levels of fat can be present in DDGS and can influence broiler performance (Swiatkiewiez and Koreleski, 2008). Alternative ingredients such as DDGS have traditionally been used in the past in poultry diets, but at relatively low levels of 5% or less in starter diets (Lumpkins et al., 2004). More recent studies have shown that positive results can be achieved when including DDGS at higher levels in later phases (Wang et al., 2007). There have been multiple reports of variability in DDGS composition with different sources varying considerably in energy, dry matter content, fat, crude protein, amino acid concentration, and availability (Cromwell et al., 1993; Spichs et al., 2002; Lumpkins and Batal, 2005; Batal and Dale, 2006; Stein et al., 2006). Increasing levels of DDGS in the feed increases dietary NSP which provides a source of potential energy. Distillers' dried grains with solubles contain 16% cellulose, 8% xylans, and 5% arabinans (Swiatkiewiez and Koreleski, 2008). The combination of nutrient variability and increased NSP content in DDGS could potentially reduce nutrient digestibility and negatively impact broiler performance.

In addition, by-products are not the only ingredients that are known to have variable nutrient content.

Nutrient Variability of Corn. Corn is a primary source of energy in broiler starter diets at approximately 65% and contributes about 20% of the protein (Cowieson, 2005). Understanding the factors that influence the energy value of corn is critical for capitalizing on the nutritive value of the grain. The corn endosperm (Figure 2-6), which is a starch-protein matrix, is crucial for influence on the nutritional value of corn.

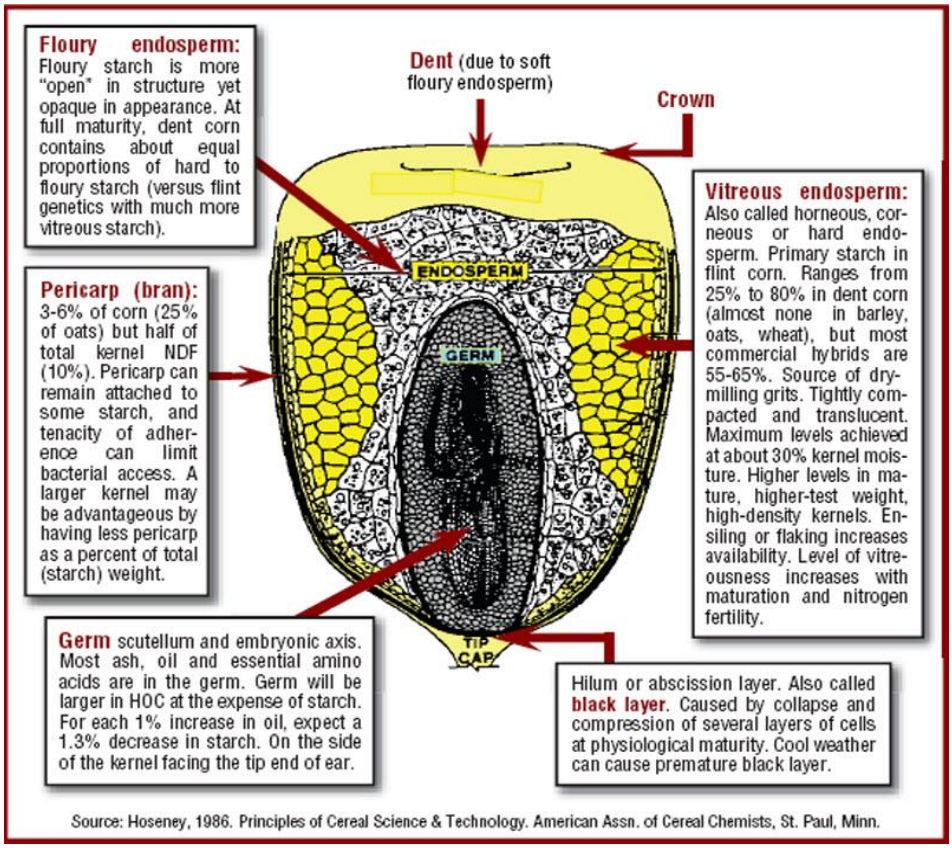


Figure 2-6. Physical properties of corn (Hoseney, 1986).

There are two types of corn endosperm; floury and vitreous. The floury and vitreous textures are a result of differences in protein and starch interactions (Rooney and Pflugfelder, 1986; Cowieson, 2005; Gayral et al., 2015). Floury endosperm is more open or loose with less encapsulation of nutrients by proteins compared to the vitreous endosperm which is hard and tightly compacted (Rooney and Pflugfelder, 1986; Cowieson, 2005; Gayral et al., 2015). Increased vitreousness is negatively related to starch digestibility with starch being located within the corn endosperm. As mentioned previously, starch digestibility in corn is a challenge due to starch being embedded in the protein matrix causing protein-starch interactions (Rooney and Pflugfelder, 1986; Cowieson, 2005; Gayral et al., 2015). Corn nutrient value varies in terms of composition and variety from year to year. Multiple factors influence corn nutrient value such as geographical location, agronomic conditions, post-harvest processing, and storage conditions (Gehring et al., 2012). Coweison et al., (2005) reported that differences in corn samples can yield variability in AME of more than 400 kcal/kg. The potential amount of variability in a primary ingredient could be detrimental to broiler performance and profitability. Knowing the nutritive value of ingredients for accurate diet formulation is of the utmost importance for poultry producers.

Near Infrared Reflectance. The ability to accurately predict the nutritive value of ingredients in real time prior to diet formulation is advantageous for poultry nutritionists. Near infrared reflectance (NIR) is commonly used in the poultry industry for the prediction of proximate analysis and AA content on numerus ingredients. The use of NIR technology has increased over the last several years and has gained much

creditability. The primary attractiveness of NIR technology is the rapid results and the relatively low costs. The NIR can be used for several parameters with calibrations for crude protein, fat, fiber, ash, moisture, among several other analyses. These calibrations are derived from a large database of ingredient profiles from different geographical locations. Although NIR technology has the ability to predict nutritive values for several ingredients, prediction of AME is a greater challenge. Although predicting the AME of corn is difficult, there are several calibrations available to predict corn AME. These calibrations predict corn AME by using parameters such as proximates, vitreousness, protein solubility index, and moisture. To test this theory, two trials were conducted evaluating corn form six different geographical locations (Masey O'Neill et al., 2013, 2014). Results from these studies indicated there was a linear relationship between the predicted AME value of corn using NIR calibration and the observed FCR, thus demonstrating an accurate prediction of corn AME value associated with broiler performance. From these studies, it is suggested that using the prediction equation reflects more accurate nutrient content prior to diet formulation allowing for more efficient diet formulation. Predicting the AME of corn is a challenge and a relatively new concept with NIR technology. Recent data suggests that accurate results are achieved if using the proper equation (Masey O'Neill et al., 2013, 2014). It is further suggested that if an accurate corn AME can be predicted using NIR, potentially xylanase response may also be predicted.

Summary. The poultry industry is continuously evolving in an effort to meet consumer demands while maintaining efficiency of production relative to cost and

performance. With this continued evolution, the need for current and informative research is continuously in high demand. Feed costs are a major area of concern for poultry producers, therefore having current strategies available that will ultimately reduce production costs is imperative. The goal of this research was to evaluate multiple strategies including exogenous enzyme inclusion, determination of methionine requirement, and use of technology to accurately predict nutritive values of ingredients.

CHAPTER III

EFFECT OF β-MANNANASE INCLUSION ON GROWTH PERFORMANCE, ILEAL DIGESTIBLE ENERGY, AND INTESTINAL VISCOSITY OF MALE BROILERS FED A REDUCED ENERGY DIET[*]

Introduction

Dietary non-starch polysaccharides (NSPs) can be found in many cereal grains such as corn and soybean meal which are common ingredients in commercial broiler diets. Dietary NSPs are mostly indigestible by monogastric animals. However, NSPs can be utilized with the addition of enzymes (Meng et al., 2005), representing a potential previously unused energy source. Corn contains minimal amounts of soluble NSP and 8% insoluble NSP, consisting of arabinoxylans and β-glucans (Choct, 2006; Slominski, 2011). Soybean meal is a primary source of vegetable protein and contains 3% soluble NSP and 16% insoluble NSP (Irish and Balnave, 1993), consisting mainly of mannans and galactomannans (Slominski, 2011). The use of by-products such as DDGS are commonly incorporated in broiler diets due to their wide spread availability. In the past, DDGS have been included in broiler diets at 5% or less (Lumpkins et al., 2004). More recent studies have shown that positive results can be achieved with higher levels of inclusion in later phases of the diet (Wang et al., 2007). Increasing levels of DDGS in the diet increases NSP content of the diet which provides a source of potential energy.

[*] Reprinted with permission from "Effect of β-mannanase inclusion of growth performance, ileal digestible energy, and intestinal viscosity of male broilers fed a reduced energy diet" by R. E. Latham, M. Williams, K. Smith, K. Stringfellow, S. Clemente, R. Brister, and J. T. Lee, 2015. Journal of Applied Poultry Research, 25(1), 40-47, Copyright 2015 by Oxford University Press and Copyright Clearance Center.

Distillers' dried grains with solubles contain 16% cellulose, 8% xylans, and 5% arabinans (Swiatkiewicz and Koreleski, 2008). The combination of nutrient variability and amount of NSP in DDGS could potentially reduce nutrient digestibility and negatively impact broiler performance. However, inclusion of exogenous enzymes could be a viable way of improving nutrient utilization of NSP in DDGS in corn and soybean meal based diets.

The use of exogenous enzymes such as phytase and carbohydrases has increased in poultry feeds in recent years. Exogenous enzymes improve nutrient utilization from cereal grains which are commonly used in broiler diets. Research has shown the inclusion of carbohydrases reduces FCR, increases nutrient digestibility, and reduces intestinal viscosity, which improves overall broiler performance and reduces diet cost (Bedford and Classen, 1992; Bedford and Morgan, 1996; Lazaro et al., 2003; Meng and Slominski, 2005; Choct, 2006). These improvements in nutrient utilization allow for energy reductions in diet formulation by the replacement of fat with corn (Masey O'Neill et al., 2012).

Beta-mannanase targets the galactomamnans present in the diet of which the main source is soybean meal. The inclusion of β-mannanase in broiler diets has shown to increase AMEn, body weight gain and improve feed conversion ratio (Daskiran et al., 2004; Jackson et al., 2004; Lee et al., 2005; Zangiabadi and Torki, 2010). The inclusion of β-mannanase has also shown to have beneficial immunological properties by reducing lesion development in broilers subjected to a necrotic enteritis challenge model through a combined *Eimeria* species and *Clostridium perfringens* challenge (Jackson et al.,

2003). Additional benefits have been attributed to the reduction of intestinal viscosity in diets with elevated galactomannan contents (Lee et al., 2003). Beta-mannanase inclusion improved broiler body weight and FCR in broilers fed a reduced energy diet (Williams et al., 2014). The previous mentioned benefits from β-mannanase inclusion were from β-mannanase sourced from *Bacillus lentus*. Therefore, the objective of the current experiment was to determine the effect of β-mannanase inclusion, sourced from *Bacillus subtilis*, on growth performance, intestinal viscosity, and ileal digestible energy in broilers fed a commercial type reduced energy diet containing DDGS and phytase.

Materials and Methods

Experimental Design. The effect of β-mannanase inclusion on broiler growth performance in a reduced energy diet was evaluated in a completely randomized block design with 3 dietary treatments during a 49 day grow-out. The experimental design consisted of a positive control diet (PC), a negative control (NC) diet with a reduced AME level of 97 kcal/kg, and a NC diet with the inclusion of β-mannanase[1].

Experimental Diets. Diets were corn and soybean meal based and formulated to be isonitrogenous (Table 3-1). Increasing levels of DDGS were included in the diet; 5% in the starter, 7.5% in the finisher and 10% in the withdrawal. The PC diet was formulated to represent a typical industry diet while the NC diet was formulated with a 97 kcal/kg AME reduction as compared to the PC. The third diet was the NC diet with the inclusion of β-mannanase at 400,000 U/kg. Pelleting temperatures were maintained

[1] CIBENZA® DE200, Novus International, St. Louis, MO

between 74˚ and 76˚ C. Lower pelleting temperatures were targeted to ensure the maximum level of enzyme integrity was achieved. The starter diet was fed as a crumble with the remaining diets fed as a pellet. A starter diet was fed at a rate of 0.45 kg/bird, finisher at 1.81 kg/bird, with the remainder of the feeding period consisting of the withdrawal diet. A minimum of 0.5% supplemental fat was maintained in the NC diets. Titanium dioxide was included in the finisher and withdrawal phases as an indigestible marker. Nutrient analysis and enzyme activity in the finished feeds was verified by the enzyme manufacturer to ensure that assayed levels recovered in the finished diet were within an acceptable range. Crude protein was determined by Association of Official Agriculture Chemists (AOAC) by combustion (AOAC 990.03) and an ether extraction to determine crude fat (AOAC 920.39).

Table 3-1. Experimental diets and calculated nutrient content for the positive control, negative control, and negative control+ β-mannanase[1] diets fed to male broilers during the starter, grower, and finisher phases.

Ingredient Profile	Starter %		Finisher %		Withdrawal %	
	PC	NC	PC	NC	PC	NC
Corn	55.82	58.33	61.39	64.08	64.83	67.36
Soybean Meal (48%)	29.79	29.48	23.31	22.79	17.72	17.47
Meat & Bone Meal	4.43	4.40	2.82	2.77	2.32	2.29
Dried Distillers' Grains with Solubles[2]	5.17	5.17	7.65	7.61	10.24	10.24
Fat – A/V Blend	2.67	0.52	2.65	0.51	2.66	0.51
Limestone	0.69	0.70	0.81	0.92	0.91	0.91
DL - methionine	0.29	0.28	0.22	0.21	0.17	0.15
Phytase[3]	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
L-lysine HCl	0.21	0.20	0.24	0.23	0.25	0.24
Sodium Chloride	0.22	0.22	0.20	0.20	0.20	0.20
Sodium Bicarbonate	0.16	0.16	0.19	0.19	0.19	0.19
L-Threonine	0.07	0.06	0.06	0.04	0.06	0.04
Trace Mineral Px[5]	0.05	0.05	0.05	0.05	0.05	0.05
BioCox[6]	0.05	0.05	0.05	0.05	0.05	0.05
Choline Chloride (60%)	0.11	0.11	0.09	0.08	0.03	0.03
Calculated Nutrient Content %						
AME (Kcal/kg)	3113	3015	3183	3086	3239	3141
Crude Protein (%)	22.91	22.92	20.21	20.11	18.41	18.43
Calcium	0.96	0.96	0.81	0.84	0.77	0.77
Avail Phosphorus	0.46	0.45	0.39	0.39	0.37	0.37
Sodium	0.19	0.19	0.18	0.18	0.18	0.18
Crude Fat	5.50	3.47	5.59	3.56	5.78	3.75
Digestible Lysine	1.22	1.21	1.06	1.04	0.93	0.92
Digestible Methionine	0.62	0.61	0.52	0.51	0.45	0.44
Digestible TSAA	0.95	0.94	0.83	0.81	0.74	0.73
Digestible Tryptophan	0.22	0.22	0.19	0.18	0.16	0.16
Digestible Threonine	0.79	0.78	0.69	0.67	0.62	0.61
Digestible Isoleucine	0.82	0.82	0.71	0.71	0.63	0.63
Digestible Valine	0.95	0.95	0.83	0.83	0.76	0.76
Digestible Cystine	0.33	0.33	0.30	0.31	0.28	0.29
Digestible Arginine	1.34	1.34	1.13	1.12	0.98	0.98
Analyzed Nutrient Content %						
Crude Protein	23.00	21.92	20.04	20.71	17.91	18.26
Crude Fat	5.84	4.12	6.14	4.23	5.65	3.15

[1] CIBENZA® DE200 – Novus International, St. Charles, MO (453.6 g/ton; 400,000 U/kg). Analyzed enzyme recovery was 0.51 U/g for starter, 0.59 U/g for finisher, and 0.54 U/g for withdrawal

[2] Dried Distillers' Grains with Solubles – moisture 8.55%; crude protein 31.8%; crude fat 7.57%; crude fiber 6.2%; ash 4.69%; total sulfur 1.04%; total phosphorus 0.93%; total calcium 0.08%

[3] Optiphos® PF – Huvepharma Inc. – Peachtree City, GA

[4] Vitamin premix added at this rate yields per kg diet 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin. The carrier is ground rice hulls

[5] Trace mineral premix added at this rate yields per kg of diet 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

[6] Active drug ingredient salinomycin sodium, 60 g/lb of activity Huvepharma Inc. – Peachtree City, GA (60 g/ton inclusion). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

Animals and Management Practice. On day of hatch, 1,800 male broiler chicks were randomly allotted to floor pens and dietary treatments based on initial BW. The study consisted of a total of 45 pens with 15 blocks of three contiguous pens with each pen containing 40 chicks. The pens were 3.34 m^2 with nipple drinkers and a tube feeder. Feed and water were available *ad libitum*. Chicks were provided age appropriate supplemental heat and were subjected to an industry type lighting program consisting of d 1 to 8, 24 h of light at 2 foot candles, d 9 to 18, 16 h of light at 0.75 foot candle, d 19 to 32, 18 h of light at 0.1 foot candle and, d 33 to termination, 20 h of light at 0.05 foot candle. Broilers were reared on fresh pine shavings. All broilers and feed were weighed by pen once per week (d 7, 14, 21, 28, 35, 42, and 49) to determine average BW, mortality adjusted FCR, FC, and cFCR. All animal husbandry procedures were conducted in accordance with an approved animal use protocol Institutional Animal Care and Use Committee (IACUC).

Digestibility Measurements. Ileal digestible energy, and intestinal viscosity measured in centipoise (cP) were determined on d 17 (5 birds per replicate pen) and d 37 (3 birds per replicate pen). Ileal content samples were pooled and then divided into two aliquots. One was used for the determination of IDE and one was used to determine ileal digest viscosity. Titanium concentration was determined via protocol outlined by Short and colleagues (1996). For this procedure, a half gram of each dried sample was weighed and ashed. Following ashing, each sample was titrated with 10 mL of sulfuric acid (7.4 M) and then boiled at 200°C for 2 h until dissolved. Samples were then titrated with 20 mL of 30% hydrogen peroxide, and brought to 100 mL final volume

using distilled water. Samples were then analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis (Model 10S UV-Vis) Spectrophotometer at 410 nm. Ileal digestible energy was calculated using the following equation (Scott et al., 1982):

Gross Ef – Excreta Ei where Excreta Ei = GE x (Tif¬/Tii)

To determine intestinal viscosity, samples were centrifuged at 3500 X g for 10 min (Lee et al., 2003a). Viscosity was determined by adding 0.5 mL of supernatant into a Brookfield Cone and Plate Viscometer[2] with a cP-40 spindle at 12 rpm at 40˚ C to represent the internal temperature of a chicken. Viscosity readings were recorded after 30 s.

Statistical Analysis. All data were subject to a one-way ANOVA using the General Linear Model (GLM) (SPSS software). Means deemed significantly different at a p-value of $p \leq 0.05$ and were further separated by Duncan's multiple range test.

Results

Body Weight. The PC fed broilers maintained a heavier average BW when compared to the NC from d 14 to d 42 indicating that the reduction in energy was sufficient to reduce average BW (Table 3-2). The inclusion of β-mannanase to the NC diet allowed broilers to achieve similar performance compared to the PC from d 7 to d 42. Beta-mannanase inclusion resulted in elevated BW compared to the NC diet from d 7 to d 42. On d 49, no significant difference was observed in average BW among all treatments.

[2] Brookfield Engineering Laboratories, DV2T Cone and Plate Viscometer, Middleboro, MA

Table 3-2. Average body weight, weekly mortality corrected feed conversion ratio, cumulative mortality corrected feed conversion ratio, ileal digestible energy (kcal/kg), and intestinal viscosity (cP) of male broilers fed a low energy diet (negative control) supplemented with a β-mannanase (negative control+β-mannanase) and compared to a high energy reference diet (positive control).

	PC	NC	NC+β-mannanase	P-value	PSEM
Body Weight					
d7 (g)	172.7[ab]	169.1[b]	174.8[a]	0.031	0.8
d14 (g)	450.1[a]	432.8[b]	451.6[a]	0.002	2.4
d21 (g)	945.2[a]	904.4[b]	939.9[a]	<0.001	4.6
d28 (kg)	1.548[a]	1.478[b]	1.526[a]	<0.001	0.007
d35 (kg)	2.191[a]	2.119[b]	2.181[a]	0.002	0.009
d42 (kg)	2.945[a]	2.853[b]	2.917[a]	0.006	0.012
d49 (kg)	3.632	3.526	3.563	0.158	0.022
FCR					
d1-7	1.090[b]	1.158[a]	1.091[b]	<0.001	0.007
d8-14	1.258[b]	1.319[a]	1.261[b]	<0.001	0.007

Table 3-2 Continued.

	PC	NC	NC+β-mannanase	P-value	PSEM
d15-21	1.405	1.441	1.437	0.082	0.007
d22-28	1.536	1.582	1.579	0.086	0.009
d29-35	1.748	1.723	1.732	0.619	0.010
d36-42	1.846	1.892	1.863	0.608	0.017
d43-49	2.110	2.127	2.213	0.481	0.037
Cumulative FCR					
d1-14	1.203[b]	1.266[a]	1.205[b]	<0.001	0.005
d1-21	1.310[b]	1.359[a]	1.326[b]	<0.001	0.005
d1-28	1.393[c]	1.441[a]	1.418[b]	<0.001	0.003
d1-35	1.492[c]	1.522[a]	1.507[b]	0.001	0.003
d1-42	1.573[b]	1.608[a]	1.587[b]	0.001	0.003
d1-49	1.661[b]	1.693[a]	1.682[ab]	0.034	0.005
IDE					
d17	3364	3306	3361	0.746	28
d37	3521[a]	3365[b]	3328[b]	0.003	22
cP					
d17	1.36	1.24	1.24	0.833	0.519
d37	1.66	1.75	1.95	0.07	0.07

[a-c] Means within a row with different superscripts differ at $p < 0.05$

[1] CIBENZA® DE200 – Novus International, St. Charles, MO (453.6 g/ton; 400,000 U/kg). Analyzed enzyme recovery was 0.51 U/g for starter, 0.59 U/g for finisher, and 0.54 U/g for withdrawal

Mortality Adjusted Feed Conversion Ratio. The PC fed broilers maintained a lower mortality adjusted FCR when compared to the NC for weeks 1 and 2 (Table 3-2). In week 1 and 2, the inclusion of β-mannanase resulted in lower FCR as compared to the NC, but was similar to the PC. No differences were observed in all subsequent weeks. Feed consumption was monitored on a weekly basis with no differences observed throughout the trial (data not shown).

Cumulative Mortality Adjusted Feed Conversion Ratio. The PC fed broilers had a lower cFCR when compared to the NC throughout the duration of the trial (Table

3-2). Reductions in cFCR were observed with the inclusion of β-mannanase as compared to the NC diet through d 42. Inclusion of β-mannanase through d 21 produced similar results to the PC. For d 1 to 35, the inclusion of β-mannanase resulted in a lower cumulative FCR when compared to the NC, however, did not reach the level of the PC. Cumulative FCR through d 42 followed a similar trend to d 21 with β-mannanase inclusion improving FCR compared to the NC, reaching similar levels to the PC. Inclusion of β-mannanase resulted in a similar cumulative FCR when compared to the PC for d 1 to 49, although it was not statistically different than the NC.

Ileal Digestible Energy. No differences were observed for d 17 IDE, however, digestible energy was increased by 55 kcal/kg in the NC with the addition of β-mannanase as compared to the NC and was within 3 kcal of the PC (Table 3-2). The increase in d 17 IDE could be responsible for improvements in growth as FCR was similar to the PC on d 21. On d 37, the PC had significantly higher IDE when compared to the NC. No effects were observed in IDE on d 37 with β-mannanase inclusion. Thus, may be related to the lower percentage of soybean meal in the diet during the withdrawal phase.

Intestinal Viscosity (cP). No differences in intestinal viscosity were observed on either day that viscosity was measured. However, viscosity values in the PC and NC diets were low and may have prevented accurate evaluation of viscosity mediation of the enzyme based on the lack of viscosity associated ingredients (Table 3-2).

Discussion

Supplementation of exogenous enzymes in poultry diets to improve nutrient utilization is a widely accepted practice within the industry. Beta-mannan is commonly found in several poultry feedstuffs, including soybean meal, palm kernel meal, copra meal, and sesame meal (Dierick, 1989). Diets containing β-mannan have shown to negatively impact animal performance by compromising weight gain and feed conversion (Anderson and Warnick, 1964), as well as reducing glucose and water absorption (Rainbird et al., 1984). It has been well documented (Lee et al., 2003; Daskiran et al., 2004; Jackson et al., 2004) that corn-SBM diets with the inclusion of β-mannanase have shown improvements in performance and enzymatic degradation of β-mannan. Results from the current study demonstrate the negative impacts of reducing energy in the diet (97 kcal/kg) on overall broiler performance, with the PC diet consistently outperforming the NC diet. The inclusion of β-mannanase in the reduced energy diet recovered the decreased caloric value throughout the trial, yielding results similar to the PC. Similar results of improved broiler performance as observed in this trial have been reported in multiple publications (McNaughton et al., 1998; Jackson et al., 2004; Tahir et al., 2005; Zou et al., 2006).

A significant increase in average male broiler BW was observed through d 42 with inclusion of β-mannanase in the NC diet as compared to the non-supplemented NC diet. Similar results in body weight gain were reported by Jackson and co-workers (2004); McNaughton and colleagues (1998); Tahir and colleagues (2005); and Zou and co-workers (2006). The significant impact of β-mannanase inclusion on broiler BW was

not observed on d 42 of the trial which could be related to lower amounts of soybean meal in the withdrawal phase of the trial.

Inclusion of β-mannanase had a significantly lower FCR when compared to the NC diet early in the trial. Jackson and co-workers (2004) reported similar results with observed improvements in FCR early in grow-out from 0 to 3 weeks with β-mannanase inclusion, maintaining these reductions throughout the trial. However, Zou and colleagues (2006) reported broilers fed a corn/soy diet had no differences in 0 to 3 week FCR, but observed improvements from week 4 to 6.

Increased intestinal viscosity has shown to have negative effects on nutrient utilization with high viscous ingredient diets. In the current study, no differences were observed in intestinal viscosity on d 17 or d 37 between treatments. Corn-SBM diets have not typically been considered highly viscous, although, SBM does contain galactomannans which are associated with elevated levels of viscosity when present in significant amounts. This observation was similar to results reported by Lee and co-workers (2003) with no significant impact on intestinal viscosity among the control diets which were corn-SBM regardless of β-mannanase inclusion.

When evaluating the effect of β-mannanase on IDE, a 55 kcal/kg difference was observed on d 17, although it was not statistically significant; however, this increase may be partially responsible for the improvement in growth performance observed early during the grow-out period. No influence of β-mannanase was observed on IDE on d 37 that may be related to a lower substrate level with the reduction of SBM percentage in the withdrawal diet. The inconsistent response of intestinal viscosity and IDE indicates

the potential for an additional reason for the improvements in growth performance. These improvements may be related to the immunological benefits of β-mannanase demonstrated in previous research by Jackson and co-workers (2004) and Jackson and co-workers (2003). Mannans are components of the surface of multiple types of pathogens which include fungi, bacteria, and viruses (Hsiao et al., 2006). Jackson and co-workers (2004) demonstrated that β-mannan can stimulate the innate immune response, potentially leading to unnecessary energy expenditure (Hsiao et al., 2006), which has been termed a feed induced immune response. Beta-mannan presence stimulates the innate immune response leading to increasing proliferation of macrophages and monocytes resulting in cytokine production (Jackson et al., 2004; Hsiao et al., 2006). Jackson and co-workers (2004) observed that reducing the amount of β-mannan content within the intestine resulted in less energy being used for innate immunity, ultimately leading to more efficient nutrient utilization and energy expenditure. Zou and colleagues (2006) also reported immunological benefits with the addition of β-mannanase increasing relative immune organ weights, IgM serum concentration, and proliferation of T-lymphocytes. However, these parameters were not evaluated in the current study.

CHAPTER IV

EFFICACY OF β-MANNANASE ON BROILER GROWTH PERFORMANCE AND ENERGY UTILIZATION IN THE PRESENCE OF INCREASING DIETARY GALACTOMANNAN

Introduction

Cereal grains are common ingredients used in standard U.S. commercial broiler diets, and these grains contain dietary non-starch polysaccharides (NSPs). Dietary NSPs are indigestible by poultry, but represent a potential energy source that can be utilized with the addition of enzymes (Meng et al., 2005). Soybean meal (SBM) is a primary source of vegetable protein and contains 3% soluble NSP and 16% insoluble NSP (Irish and Balnave, 1993). These NSP consist mainly of mannans and galactomannans (Slominski, 2011). Beta-mannan, or galactomannan, is a polysaccharide that has repeating units of mannose containing galactose and/or glucose (Carpita and McCann, 2000; Hsiao et al., 2006). Galactomannan content in dehulled SBM has been reported at levels from 1.02 to 1.51% (Hsiao et al., 2006), but little information exists on galactomannan content in SBM. Although galactomannan content of SBM is in low concentration, it is a concern for nutritionists because it has anti-nutritive properties. Mannans are surface components of multiple pathogens and the innate immune system reacts to antigens on these pathogens. Mannans in the diet can stimulate the innate immune system and lead to a purposeless energy utilizing immune response (Hsiao et al., 2006). Inclusion of exogenous enzymes could be a viable option to mitigate some of these negative effects in diets containing SBM.

Specifically, the enzyme β-mannanase targets galactomannan present in the diet of which the main source is SBM. The inclusion of β-mannanase in broiler diets has shown to increase AME, BWG and improve FCR (Daskiran et al., 2004; Jackson et al., 2004; Lee et al., 2005; Zangiabadi and Torki, 2010).

Galactomannan in guar gum has a similar galactose:mannose ratio to SBM with a 0.1% difference (Whistler and Smart, 1953; Whistler and Saarnio, 1957; Hsiao et al., 2006). Beta-mannanase inclusion has been reported to have immunological benefits. When including β-mannanase into broiler diets, reductions in lesion development were observed in broilers subjected to a necrotic enteritis model through a combined *Eimeria* species and *Clostridium perfringens* challenge (Jackson et al., 2003). Additional benefits have been attributed to the reduction of viscous material in diets with elevated galactomannan contents (Lee et al., 2003a). Beta-mannanase inclusion has also shown to increase average BW and reduce FCR in broilers fed a reduced energy diet (Williams et al., 2014).

Benefits from β-mannanase inclusion in broiler diets has been well documented (Jackson et al., 2003; Lee et al., 2003a; Daskiran et al., 2004; Jackson et al., 2004; Lee et al., 2005; Zangiabadi and Torki, 2010), as well as the need for increasing the level of substrate available for β-mannanase. However, the level of β-mannanase in combination with galactomannan concentrations required to achieve maximum broiler performance is not known. Therefore, the objective of the current experiment was to investigate the impact of β-mannanase inclusion on growth performance, intestinal viscosity, and IDE in broilers fed diets varying in galactomannan concentrations.

Materials and Methods

Experimental Design. The effect of β-mannanase[3] inclusion on broiler growth performance and energy utilization when administered in the presence of various dietary galactomannan[4] (GM) concentrations was evaluated in a completely randomized block design during a 42 d grow-out. The experimental design consisted of a 3 (β-mannanase - 0, 200, or 400 g/ton) X 3 (GM - 0, 1500, or 3000 ppm) factorial yielding a total of 9 treatments.

Experimental Diets. Diets were corn-SBM based and formulated in an effort to reduce SBM content to ultimately decrease the amount of GM content in the control diet (Table 4-1). Therefore, corn gluten, DDGS, and meat and bone meal (MBM) were included in the diet as alternative protein sources. The guar gum used in the current trial had a galactomannan concentration of 70% and was included to increase the level of β-mannan substrate. The analyzed nutrient content for crude protein was 22.50% for the starter, 20.10% for the grower, and 18.84% for the finisher. For each feeding phase, a large control diet was manufactured and split into 9 equal treatments. Treatments consisted of β-mannanase at 0, 200, or 400 g/ton and GM at 0, 1500, or 3000 ppm. Sand was used as a filler to ensure all inclusions were equal. Pelleting temperatures were maintained between 74˚ and 76˚ C. Lower pelleting temperatures were targeted to ensure the maximum level of enzyme activity in the finished feed. The starter diet was

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[3] Hemicell®-HT, Elanco Animal Health, Greenfield, IN

[4] PROCOL®, POLYPRO, Minneapolis, MN

fed as a crumble with the remaining diets fed as a pellet. The starter diet was fed from d 0 to 14, grower from d 15 to 28, and finisher from d 29 to 42. Titanium dioxide was included in all dietary phases as an indigestible marker to determine IDE. Nutrient analysis and enzyme activity in the finished feed was verified by the manufacturer to ensure assayed levels in the finished feed were within an acceptable range.

Table 4-1. Experimental diets and calculated nutrient content for male broilers fed a diet with varying concentrations of β-mannanase[1] and galactomannan[2] during the starter, grower, and finisher phases.

Ingredient Profile	Starter %	Grower %	Finisher %
Corn	50.28	50.49	43.95
Soybean Meal (48%)	16.02	9.61	8.00
Corn Gluten (60%)	6.00	6.00	8.00
DL - methionine	0.34	0.27	0.15
L-lysine HCl	0.59	0.64	0.57
L-Threonine	0.15	0.27	0.11
Fat – A/V Blend	3.35	4.32	5.44
Whole Wheat	10.00	12.00	20.00
Limestone	0.74	0.85	1.44
Monocalcium Phosphate	-	-	0.55
Sodium Chloride	0.15	0.15	0.18
Sodium Bicarbonate	0.25	0.25	0.33
Trace Mineral Px[3]	0.05	0.05	0.05
Vitamin premix[4]	0.25	0.25	0.25
Low Oil- Distillers Dried Grains with Solubles	6.00	10.00	10.00
Meat & Bone Meal	5.40	3.80	-
Coccidiostat[5]	0.05	0.05	0.05
Potassium Sulfate	0.37	0.59	0.53
Phytase[6]	0.01	0.01	0.01
Titanium Dioxide	0.40	0.40	0.40
Calculated Nutrient Content %			
AME (Kcal/kg)	3102	3168	3234
Crude Protein (%)	22.50	20.10	18.84
Calcium	0.95	0.83	0.77
Avail Phosphorus	0.45	0.39	0.37
Sodium	0.18	0.18	0.20

Table 4-1 Continued.

Ingredient Profile	Starter %	Grower %	Finisher %
Crude Fat	6.70	7.67	8.34
Digestible Lysine	1.22	1.08	0.93
Digestible Methionine	0.67	0.59	0.46
Digestible TSAA	0.95	0.84	0.74
Digestible Tryptophan	0.17	0.14	0.13
Digestible Threonine	0.79	0.81	0.62
Digestible Isoleucine	0.74	0.64	0.62
Digestible Valine	0.88	0.77	0.73
Digestible Cystine	0.28	0.26	0.27
Digestible Arginine	1.09	0.86	0.72

[1] Hemicell® - HT- Elanco Animal Health – Greenfield, IN

[2] PROCOL® - POLYPRO – Minneapolis, MN

[3]Trace mineral premix added at this rate yields per kg of diet 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

[4]Vitamin premix added at this rate yields per kg diet 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin. The carrier is ground rice hulls

[5]Active drug ingredient salinomycin sodium, 60 g/lb of activity Huvepharma Inc. – Peachtree City, GA (60 g/ton inclusion).). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

[6] Optiphos® – Huvepharma Inc. – Peachtree City, GA

[7] Expected β-mannanase activity 35 MMU at 200 g/ton and 70 MMU at 400g/ton with β-mannanase recovery for the starter at 36.6 MMU at 200 g/ton and 54.5 MMU at 400 g/ton; β-mannanase recovery for the grower at 25.8 MMU at 200 g/ton and 53.7 MMU at 400 g/ton; β-mannanase recovery for the finisher at 41.9 MMU at 200 g/ton and 63.5 MMU at 400 g/ton

Animals and Management Practice. On d of hatch, 3,132 male broiler chicks were randomly allotted to floor-pens and dietary treatments based on initial BW. The study consisted of 108 total pens with 12 blocks of 9 contiguous pens, each containing 29 chicks. The pens were 1.672 m^2 floor pens with nipple drinkers and a tube feeder. Feed and water were available *ad libitum*. Chicks were provided age appropriate supplemental heat and were subjected to an industry type lighting program consisting of d 1 to 8, 24 h of light at 2 foot candles, d 9 to 18, 16 h of light at 0.75 foot candle, d 19 to

32, 18 h of light at 0.1 foot candle and, d 33 to termination, 20 h of light at 0.05 foot candle. Broilers were reared on used litter that was top-dressed with fresh pine shavings. All broilers and feed were weighed by pen on d 14, 28, and 42 to determine average BW, mortality adjusted FCR, FC, and cFCR. Ileal digestible energy and intestinal viscosity measured in cP were determined on d 14 (5 birds per replicate pen), d 28 (4 birds per replicate pen), and d 42 (3 birds per replicate pen). All animal husbandry procedures were conducted in accordance with an approved animal use protocol (IACUC).

Digestibility Measurements. Ileal samples were pooled, homogenized and divided into two aliquots with one used for the determination of IDE and one used to determine intestinal viscosity. For IDE determination, samples were dried at 100˚ C for 24 h and gross energy of feed and ileal digesta were determined using a Parr 6400 bomb calorimeter[5]. Titanium concentration was determined via protocol outlined by Short and colleagues (1996). Procedures as described in Chapter III.

Statistical Analysis. All data were subject to a 3X3 factorial ANOVA using the GLM model (SPSS software) with main effect means deemed significantly different at $p \leq 0.05$. Means deemed significantly different were further separated by Duncan's Multiple Range Test. In the case of an interaction, data were subject to a One-Way ANOVA with means deemed significantly different at $p \leq 0.05$ and as with main effect means, means deemed significantly different were further separated by Duncan's Multiple Range Test.

[5] Parr Instrument Company, 6400 Bomb Calorimeter, Moline, IL

Results

Body Weight. Galactomannan levels impacted BW on d 14 in broilers fed the control and low inclusion level, yielding a suppressed BW when compared to high level of GM inclusion (Table 4-2). An interaction ($p=0.024$) was present between β-mannanase level and GM on d 28 regarding average BW. In broilers fed diets without β-mannanase, increasing levels of GM resulted in a decrease in average male BW. Inclusion of β-mannanase at 200 and 400 g/ton in diets containing high supplemental GM increased d 28 BW compared to diets without β-mannanase. At the conclusion of the trial on d 42, the inclusion of β-mannanase or supplemental GM did not impact average BW.

Feed Consumption. No differences were observed in average FC for the starter and finisher phases with the inclusion of β-mannanase or supplemental GM (Table 4-2). During the grower phase, broilers fed diets containing the highest level of GM yielded an increase in FC when compared to no supplemental GM, with the low level of GM yielding intermediate results.

Table 4-2. Average body weight and dietary phase feed consumption of male broilers fed a diet with varying concentrations of β-mannanase and galactomannan to 14, 28, and 42 days of age.

	g/ton	ppm	Body Weight			Feed Consumption (bird/day)		
TRT	β-mannanase	Galactomannan	Day 14 (kg)	Day 28 (kg)	Day 42 (kg)	Starter (kg)	Grower (kg)	Finisher (kg)
1	0	0	0.403	1.425[a]	2.459	0.034	0.118	0.163
2	0	1500	0.405	1.368[b]	2.392	0.034	0.120	0.161
3	0	3000	0.390	1.295[c]	2.356	0.033	0.120	0.169
4	200	0	0.410	1.423[a]	2.370	0.034	0.117	0.158
5	200	1500	0.410	1.414[ab]	2.386	0.034	0.119	0.160
6	200	3000	0.399	1.382[ab]	2.409	0.033	0.122	0.165
7	400	0	0.406	1.408[ab]	2.413	0.034	0.118	0.160
8	400	1500	0.402	1.402[ab]	2.351	0.033	0.117	0.156
9	400	3000	0.402	1.386[ab]	2.378	0.034	0.121	0.161
Main Effect Means								
β-mannanase								
0			0.399	1.363	2.402	0.033	0.119	0.164
200			0.406	1.406	2.389	0.034	0.119	0.161
400			0.403	1.399	2.381	0.034	0.118	0.159
	Galactomannan							
-	0		0.406[a]	1.419	2.414	0.034	0.117[b]	0.160
-	1500		0.406[a]	1.395	2.377	0.034	0.118[ab]	0.159
	3000		0.397[b]	1.355	2.381	0.033	0.121[a]	0.165
	p-value							
β-mannanase			0.231	0.005	0.880	0.633	0.749	0.466
Galactomannan			0.037	<0.001	0.632	0.202	0.045	0.325
β-mannanase x Galactomannan			0.667	0.024	0.717	0.248	0.514	0.979
PSEM			0.002	0.007	0.021	<0.001	<0.001	0.002

[a-c] Means within a column with different superscripts differ at $p < 0.05$

[1] Hemicell® - HT- Elanco Animal Health – Greenfield, IN

[2] PROCOL® - POLYPRO – Minneapolis, MN

Mortality Adjusted Feed Conversion Ratio. Inclusion of β-mannanase or supplemental GM did not impact mortality adjusted FCR during the starter and finisher phases (Table 4-3). During the grower phase, an interaction ($p<0.001$) was present between β-mannanase and GM content. Broilers fed diets without β-mannanase had an increase in FCR with increasing levels of GM. In broilers fed diets containing β-mannanase at 200 g/ton and 400 g/ton, the high inclusion of GM resulted in an increase in mortality adjusted FCR. The inclusion of β-mannanase at 200 and 400 g/ton in diets

containing supplemental GM (low and high) reduced grower FCR compared to diets without β-mannanase.

Cumulative Mortality Adjusted Feed Conversion Ratio. An interaction between β-mannanase and GM was observed through d 0-28, d 14-42, and d 0-42 with respect to cFCR (Table 4-3). Cumulatively, through d 0 to 28, d 14 to 42, and d 0 to 42, broilers fed diets without β-mannanase had higher cFCRs as concentrations of GM increased. At the initiation of the trial through d 28, broilers fed diets with β-mannanase, regardless of concentration, had increased cFCR at the high level of GM inclusion when compared to diets containing the low level of GM inclusion and the control. In diets containing supplemental GM inclusion of β-mannanase at 200 and 400 g/ton reduced cFCR for d 0 to 28 compared to diets without β-mannanase. Galactomannan supplementation did not impact cFCR for d 14 to 42 and d 0 to 42 when included in diets containing β-mannanase at 200 g\ton. However, broilers fed diets with the inclusion of β-mannanase at 400 g/ton and the high concentration of GM, had increased cFCR when compared to no GM supplementation on d 14-42. From d 14 to 42, inclusion of β-mannanase at 200 g/ton in diets containing high levels of GM reduced cFCR compared to diets without β-mannanase. At the conclusion of the trial (d 0 to 42), increased cFCR was observed in diets containing β-mannanase at 400 g/ton and high levels of GM supplementation compared to the low concentration of GM and the control. Inclusion of β-mannanase at 200 and 400 g/ton in diets containing high levels of GM reduced cFCR through d 42 compared to diets without β-mannanase.

Table 4-3. Feed conversion ratio and cumulative feed conversion ratio of male broilers fed a diet with varying concentrations of β-mannanase and galactomannan to 14, 28, and 42 days of age.

	g/ton	ppm	Dietary Phase FCR			Cumulative FCR		
TRT	**β-mannanase**	**Galactomannan**	**Starter**	**Grower**	**Finisher**	**Day 0-28**	**Day 14-42**	**Day 0-42**
1	0	0	1.316	1.602^{c}	2.214	1.515^{c}	1.865^{d}	1.757^{e}
2	0	1500	1.303	1.700^{b}	2.219	1.573^{b}	1.932^{bc}	1.805^{bcd}
3	0	3000	1.351	1.799^{a}	2.224	1.653^{a}	1.998^{a}	1.868^{a}
4	200	0	1.311	1.604^{c}	2.343	1.513^{c}	1.912^{bcd}	1.788^{cde}
5	200	1500	1.313	1.637^{c}	2.287	1.537^{c}	1.927^{bc}	1.795^{bcd}
6	200	3000	1.305	1.699^{b}	2.261	1.576^{b}	1.934^{b}	1.812^{bc}
7	400	0	1.310	1.635^{c}	2.219	1.536^{c}	1.887^{cd}	1.774^{de}
8	400	1500	1.308	1.623^{c}	2.323	1.527^{c}	1.916^{bc}	1.793^{cde}
9	400	3000	1.324	1.700^{b}	2.298	1.583^{b}	1.957^{ab}	1.830^{b}
Main Effect Means								
β-mannanase								
0			1.324	1.700	2.219	1.580^{a}	1.932	1.810
200			1.309	1.647	2.297	1.542^{b}	1.926	1.798
400			1.314	1.653	2.280	1.549^{b}	1.920	1.799
Galactomannan								
-	0		1.312	1.614	2.259	1.521^{c}	1.888^{c}	1.773^{c}
-	1500		1.308	1.654	2.276	1.545^{b}	1.925^{b}	1.797^{b}
	3000		1.327	1.733	2.261	1.604^{a}	1.965^{a}	1.837^{a}
	p-value							
β-mannanase			0.349	<0.001	0.107	<0.001	0.681	0.382
Galactomannan			0.160	<0.001	0.884	<0.001	<0.001	<0.001
β-mannanase x Galactomannan			0.239	<0.001	0.397	<0.001	0.033	0.011
PSEM			0.004	0.008	0.019	0.006	0.007	0.0

[a-e] Means within a column with different superscripts differ at $p < 0.05$

[1] Hemicell® - HT- Elanco Animal Health – Greenfield, IN

[2] PROCOL® - POLYPRO – Minneapolis, MN

Ileal Digestible Energy. On d 14, an interaction (p=0.002) was observed between β-mannanase and GM concentrations with respect to IDE (Table 4-4). Inclusion of β-mannanase at 400 g/ton in diets without supplemental GM yielded an increase in d 14 IDE compared to diets without β-mannanase. The inclusion of β-mannanase at 200 g/ton in diets supplemented with high levels of GM increased IDE on

d 14 compared to diets without β-mannanase and β-mannanase at 400 g/ton. In diets containing β-mannanase at 400 g/ton, supplementation of high GM reduced d 14 IDE compared to diets without supplemental GM. On d 28, increasing levels of GM did not impact IDE. However, as β-mannanase inclusion increased, there was an increase in IDE. Inclusion of β-mannanase or supplemental GM did not impact IDE at the conclusion of the trial on d 42.

Table 4-4. Ileal digestible energy and intestinal viscosity of male broilers fed a diet with varying concentrations of β-mannanase and galactomannan to 14, 28, and 42 days of age.

	g/ton	ppm	Ileal Digestible Energy			Viscosity (cP)		
TRT	β-mannanase	Galactomannan	Day 14	Day 28	Day 42	Day 14	Day 28	Day 42
1	0	0	3133^{b}	3219	3130	13.16^{e}	1.98^{d}	2.33
2	0	1500	3306^{ab}	2959	3246	19.30^{bc}	3.95^{bc}	2.52
3	0	3000	3126^{b}	3010	3272	28.79^{a}	10.03^{a}	3.15
4	200	0	3246^{ab}	3109	3291	17.37^{cd}	2.17^{d}	2.47
5	200	1500	3281^{ab}	3268	3363	14.41^{de}	3.72^{bc}	2.62
6	200	3000	3420^{a}	3126	3233	21.91^{b}	4.32^{b}	2.90
7	400	0	3440^{a}	3373	3362	13.00^{e}	2.93^{cd}	2.29
8	400	1500	3263^{ab}	3344	3256	17.82^{cd}	3.50^{bc}	2.72
9	400	3000	3119^{b}	3304	3271	19.65^{bc}	4.40^{b}	2.96
Main Effect Means								
β-mannanase								
0			3190^{b}	3051^{c}	3211	20.42^{a}	5.32^{a}	2.67
200			3313^{a}	3165^{b}	3298	17.90^{b}	3.40^{b}	2.66
400			3274^{ab}	3343^{a}	3296	16.82^{b}	3.61^{b}	2.65
	Galactomannan							
-	0		3276	3235	3259	14.51^{c}	2.36^{c}	2.36^{b}
-	1500		3284	3190	3290	17.18^{b}	3.73^{b}	2.62^{b}
	3000		3216	3137	3260	23.45^{a}	6.25^{a}	3.00^{a}
	p-value							
β-mannanase			0.052	<0.001	0.272	0.005	<0.001	0.996
β-glucan			0.444	0.353	0.933	<0.001	<0.001	<0.001
β-mannanase x β-glucan			0.002	0.060	0.267	<0.001	<0.001	0.657
PSEM			25	27	24	0.61	0.26	0.06

[a-e] Means within a column with different superscripts differ at $p < 0.05$

[1] Hemicell® - HT- Elanco Animal Health – Greenfield, IN

[2] PROCOL® - POLYPRO – Minneapolis, MN

Intestinal Viscosity (cP). An interaction ($p<0.001$) was observed between β-mannanase and GM concentrations on d 14 and d 28 with regards to intestinal viscosity (Table 4-4). On d 14 and d 28, broilers fed diets without β-mannanase had increased viscosity with increasing concentrations of GM. On d 14, diets containing β-mannanase at 200 g/ton reduced viscosity regardless of GM concentration compared to diets without β-mannanase. Beta-mannanase inclusion at 400 g/ton in high GM diets reduced viscosity compared to diets without β-mannanase. On d 28, in diets containing β-mannanase at 200 g/ton, supplemental GM (low and high) increased viscosity compared to the control. Supplemental GM (low and high) increased intestinal viscosity on d 28 compared to the control in diets supplemented with β-mannanase at 400 g/ton. On d 28, inclusion of β-mannanase at 200 and 400 g/ton in diets containing high levels of supplemental GM reduced intestinal viscosity compared to diets without β-mannanase. On d 42, supplementing diets with high levels of GM increased intestinal viscosity compared to diets without supplemental GM and low supplemental GM concentration.

Discussion

Supplementation of exogenous enzymes in broiler diets to improve performance parameters is a common practice in the poultry industry. Galactomannan is commonly found in a variety of poultry feedstuffs, including soybean meal, palm kernel meal, copra meal, and sesame meal (Dierick, 1989). Diets containing excess GM have shown to negatively impact animal performance, compromising weight gain and feed conversion (Anderson and Warnick, 1964), as well as glucose and water absorption (Rainbird et al., 1984). It has been well documented that corn/soy based diets with the inclusion of β-

mannanase have shown improvements in performance, nutrient utilization, and enzymatic degradation of GM (Lee et al., 2003a; Daskiran et al., 2004; Jackson et al., 2004). Results from the current study demonstrate negative impacts of increasing GM concentrations on overall broiler performance. Negative performance effects were exacerbated when GM content was increased. The inclusion of β-mannanase enhanced performance of broilers fed diets containing GM when compared to broilers fed diets containing GM without β-mannanase. Similar results have been observed with performance parameters negatively impacted by increased GM content (Lee et al., 2005) and performance improvements with the inclusion of β-mannanase (McNaughton et al., 1998; Jackson et al., 2004, Tahir et al., 2005; Zou et al., 2006).

A reduction in average male BW was observed through d 14 and 28 with inclusion of GM at 3000 ppm in the diet as compared to the diet with no GM inclusion and GM included at 1500 ppm. Lee and co-workers (Lee et al., 2005) observed similar results with increased levels of GM and subsequent negative impact on broiler BW. Galactomannan is a viscous additive that can severely impede broiler performance. Galactomannan inclusion primarily affected young birds which agree with other published results (Nagpal et al., 1971; Thakur and Pradhan, 1975; Verma and McNab, 1982; Patel and McGinnis, 1985). Growth inhibition with GM inclusion ceased after the grower phase with similar BW during the finisher phase. Equalized broiler BW during the finisher phase suggests that broilers adapted to dietary GM over time. Inclusion of β-mannanase to diets containing GM improved BW to levels similar to control during the grower phase. Conflicting results were reported by Lee et al., (2005) with the

inclusion of β-mannanase resulting in no differences in BW, regardless of the amount of GM in the diet. Other researchers have emphasized improvements in body weight gain in trials conducted by McNaughton and co-workers (1998); Jackson and co-workers (2004); Tahir and colleagues (2005); and Zou and colleagues (2006).

During the grower phase from d 15 to 28, an interaction ($p<0.001$) was observed between GM and β-mannanase inclusion. There was a linear increase in mortality corrected FCR as GM content increased in the diet with a 5.6% (10 point) increase in FCR at 1500 ppm and a 11.1% (20 point) increase at 3000 ppm when compared to control. Lee and colleagues (2005) reported similar observations with increased FCR when GM was added to diets containing 7.5% or more of a guar additive, regardless of type. Earlier findings reported by Thakur and Pradhan (1975) and Petel and McGinnis (1985) also support findings in the current study having increased levels of GM and a negative impact on feed efficiency. Once β-mannanase was included in the diet containing GM at 1500 ppm, FCR was reduced to levels similar to the control. Decreased feed conversion ratio was associated with β-mannanase inclusion when GM was added at 3000 ppm, compared to broilers fed the same GM inclusion level without β-mannanase. Petel and McGinnis (1985) and Lee and colleagues (2005) also observed improvements in FCR when β-mannanase was included into diets containing increased levels of GM. No difference was observed between β-mannanase inclusion rates of 200g/ton and 400 g/ton. Similar trends were observed with cumulative FCR. These results confirm the potential for β-mannanase to utilize increased levels of substrate, resulting in growth performance equivalent to standard corn-SBM diets.

Feed consumption was increased from d 15 to 28 with the high concentration of GM inclusion when compared to control. This may be attributed to increased consumption of diets with high levels of GM to meet daily nutritional requirements. Increased GM levels in the diet lead to an increased intestinal viscosity, which reduces nutrient utilization. Lee and colleagues (2005) reported alternative findings with broilers fed diets containing the highest concentration of GM consuming less feed.

It has been well documented that increased GM content in diets results in an increase in intestinal viscosity therefore impeding broiler performance (Anderson and Warnick, 1964; Burnett, 1966; Almirall et al., 1995; Choct et al., 1995; Lee et al., 2003a, 2003b). Damaging effects associated with increased viscous digesta are more prominent in younger birds as compared to older birds (Almirall et al., 1995), suggesting that broilers adapt to dietary challenges. In the current study on d 14 and 28, there was an interaction ($p<0.001$) between enzyme and GM inclusion. Intestinal viscosity increased as GM content increased in the diet. When including β-mannanase in the diets, regardless of the level, the diet containing GM at 1500 ppm resulted in no differences when compared to the diet containing GM at the same concentration without β-mannanase. However, β-mannanase inclusion in diets containing GM at 3000 ppm resulted in a reduction in intestinal viscosity compared to the diet containing GM at the same concentration without β-mannanase. This observation was similar to results reported by Lee and colleagues (Lee et al., 2003a) with β-mannanase inclusion reducing intestinal viscosity with the inclusion of various GM containing ingredients. During the final phase from d 29 to 42, intestinal viscosity was increased in the diets containing the

high concentration of GM with the diet containing the low concentration and no GM being statistically similar.

When evaluating IDE, an interaction ($p<0.002$) was present on d 14 between enzyme and GM inclusion. Overall, inclusion of β-mannanase improved IDE in diets with and without additional GM inclusion. Beta-mannanase inclusion at 200 g/ton in the diet containing the high concentration of GM increased IDE by 294 kcal/kg when compared to the diet containing GM at the same concentration without β-mannanase. However, this improvement did not continue when the inclusion of β-mannanase was increased to 400 g/ton. During the grower phase, IDE improvements were dependent on β-mannanase inclusion. As β-mannanase inclusion increased in the diet, IDE increased regardless of GM concentration. These results were supported by the improvement in BW during the same phase. In one study, Daskiran (2004) reported similar results with GM inclusion reducing nutrient digestibility while inclusion of β-mannanase mitigated the negative impact produced by increased GM concentrations. These data support alternatives such as increased levels of substrate and supplemental β-mannanase can be advantageous.

Inconsistent responses on viscosity and IDE leave room for speculation that there is an additional reason for improvements in growth performance. As previously mentioned, immunological benefits from the inclusion of β-mannanase have been reported (Jackson et al., 2003; Jackson et al., 2004; Hsiao et al., 2006; Zou et al., 2006). Mannan presence elicits an innate immune response when an immune response is not required. Jackson et al., (2004) demonstrated that mannans stimulate the innate immune

response, leading to unnecessary energy expenditure (Hsiao et al., 2006) which is considered to be a feed induced immune response. Jackson and colleagues (2004) also observed that reducing the amount of intestinal mannan content resulted in reduced feed induced immune response, ultimately leading to more efficient nutrient utilization and energy expenditure.

CHAPTER V

DETERMINATION OF THE SULFUR AMINO ACID REQUIREMENT OF THE COBB 500 MALE BROILER BETWEEN 35 AND 49 DAYS-OF-AGE

Introduction

Over the last 50 years, there have been major advances in how broilers are grown through selection and nutrition. Broiler diets are currently formulated on an ideal AA basis with the most limiting AA being Met or SAA, lysine, threonine, and valine (Kidd et al., 2004; Corzo et al., 2007; Dozier III et al., 2008; Zhang and Guo, 2008; Mejia et al., 2012). The ratio's that AA are included into broiler diets has changed over the last 2 decades from the NRC (1994) with a SAA ratio at 84% to Lesson and Summers (2005) with a SAA ratio of 88% to Lys. Recent research support a SAA range of 75 to 78%, which is much lower than the recommendations of previous years (Goulart et al., 2011; Rostagno et al., 2011). Current research availability focusing on AA requirements for the modern broiler is limited and in need of revision for more accurate diet formulation.

Sulfur AA are essential for growth, feather formation, and methyl donation (Garcia and Batal, 2005). To better balance and meet the dAA requirements of poultry, a number of synthetic amino acids are being used. The majority of AA are manufactured through fermentation with the exception of DL-Met and DL-HMTBa which are chemically synthesized. During the chemical synthetization of methionine, production begins with raw materials such as methanol and sulfur that combine to form the intermediates such as methyl mercaptan and acrolein. These intermediates are still toxic

at this stage and depending on the combination, results in the final product of DLM or HMTBa.

The chemical difference between the two Met sources is the amino group attached to the DLM molecule and the hydroxyl group attached to the HMTBa molecule (Dibner, 2003). The DLM is an amino acid and the HMTBa is an acidic analog that is converted into an AA. Both Met sources chemically synthesize the D and L form, and absorb and covert that form to L-methionine in the body. Previous research investigating the Met requirement and dSAA:dLys ratio, Zhang and Guo (2008) reported a linear decrease in feed to gain ratio as HMTBa supplementation increased in broilers from d 21 to 42 suggesting that increased levels of HMTBa inclusion improves FCR (Zhang and Guo, 2008). Goulart et al. (2011) observed that broilers from d 36 to 42 reached maximum performance at a dSAA:dLys ratio of 72%, although the requirement to maximize breast weight was achieved at dSAA:dLys ratio of 76% (Dozier III and Mercier, 2013). The majority of current data available for Met requirement has been through d 42 with minimal observations to d 49 which is a common age of broiler growth in the U.S. poultry industry. The objectives of the current experiment were to determine the dSAA requirement of the male Cobb 500 broilers from d 35 to 49 and to evaluate the bioavailability of HMTBa.

Materials and Methods

Experimental Design. The dSAA requirements of the male Cobb 500 broiler from d 35 to 49 and the bioavailability of HMTBa was evaluated in a completely randomized block design for a 14 day experimental period. The experimental design

consisted of a dose titration of DL-Met[6] to the basal diet resulting in 7 dSAA levels that ranged from 0.491 to 0.882% and 1 diet containing HMTBa[7] (88% purity; 100% biologically active) at 0.687%, yielding a total of 8 treatments (Table 5-1).

Table 5-1. Experimental treatment methionine inclusion.

TRT #	Product (Concentration; Bioavalability)	Metionine Inclusion, %	Digestible Methionine, %	Digestible Sulfur Amino Acids, % *	Digestible Sulfur Amino Acids: Digestible Lysine, %	Analysis
1	DLM[1] (99%)	0	0.248	0.491	54.6	Quadratic Broken Line
2	DLM (99%)	0.066	0.313	0.557	61.8	
3	DLM (99%)	0.132	0.379	0.622	69.1	
4	DLM (99%)	0.197	0.444	0.687	76.3	
5	DLM (99%)	0.263	0.509	0.752	83.5	
6	DLM (99%)	0.329	0.574	0.817	90.8	
7	DLM (99%)	0.395	0.639	0.882	98.0	
8	HMTBA[2] (88%; 100 BV)	0.222	0.444	0.687	76.3	Contrast Analysis

* Digestible sulfur amino acid of DL-2-Hydroxy-4-Methylthio Butanoic Acid treatments were calculated at 100% bioavailability; digestible lysine = 0.90%; digestible cystine = 0.243%

[1] DL methionine

[2] DL-2-Hydroxy-4-Methylthio Butanoic Acid

Animals and Management Practice. On d of hatch, 2,496 Cobb 500 x Cobb MX male broiler chicks were randomly allotted to floor-pens based on initial BW. The study consisted of a total of 104 pens with 13 blocks of 8 pens with each pen containing 24 chicks. The pens were 3.34 m^2 with nipple drinkers and a tube feeder. Feed and

[6] Rhodimet NP99, Adisseo USA, Alpharetta, GA

[7] Rhodimet AT88, Adisseo USA, Alpharetta, GA

water were available *ad libitum.* Chicks were provided age appropriate supplemental heat and were subjected to an industry type lighting program consisting of d 1 to 8, 24 h of light at 2 foot candles, d 9 to 18, 16 h of light at 0.75 foot candle, d 19 to 32, 18 h of light at 0.1 foot candle and, d 33 to termination, 20 h of light at 0.05 foot candle. Broilers were reared on used litter that was top dressed with fresh pine shavings. Broilers were fed common diets for the first 35 d with BW and FCR being recorded at feed changes (d 14, 24, and 35). On d 35, all pens were reduced from 24 to 21 birds per pen. Birds were weighed and re-randomized so that there was no statistical difference in pen weights and then assigned to treatments for the 14 d test period. Evaluated parameters consisted of average BW, body weight gain (BWG), mortality corrected FCR, cFCR, and FC. All animal husbandry procedures were conducted in accordance with an approved animal use protocol (IACUC).

Common Diets. Diets were corn-SBM based and formulated on an ideal AA basis to represent typical broiler diets being fed in the industry (Table 5-2). The starter was fed thru d 14, grower from 15 to 24, and finisher from d 25 to 35. Amino acids were included as a ratio to dLys and dLys levels were typical to what would be seen in standard broiler diets at 1.20%, 1.08%, and 0.98% for the starter, grower, and finisher, respectively.

Table 5-2. Common diets and calculated nutrient content for male broilers.

Ingredient Profile	Starter %	Grower %	Finisher %
Corn	48.813	54.493	59.763
Wheat	7.000	8.000	9.000
Soybean Meal (48% CP)	35.778	29.672	23.821
Meat & Bone Meal	3.000	3.500	4.000
Limestone	1.085	0.860	0.715
Monocalcium Phosphate	0.628	0.328	0.146
Copper Sulfate Pentahydrate	0.050	0.050	0.050
Sodium Chloride	0.314	0.284	0.267
Sodium Bicarbinate	0.200	0.200	0.200
L-Lysine HCl, 78.8%	0.173	0.194	0.234
DL-Methionine, 99%	0.312	0.272	0.234
L-Threonine, 98.0%	0.075	0.080	0.093
Choline 60%	0.098	0.123	0.146
Fat-A/V Blend	2.319	1.789	1.176
Phytase [E. coli 6-ase] - 2,500 FTU/gram[1]	0.020	0.020	0.020
Salinomycin[2]	0.050	0.050	0.050
Vitamin E (500 IU/g)	0.001	0.001	0.001
Corn Starch	-	-	-
Trace Mineral Premix[3]	0.059	0.060	0.060
Vitamin Premix[4]	0.025	0.025	0.025
Calculated Nutrient Content %			
AME (kcal/kg)	3,031	3,085	3,125
Crude Protein (%)	23.48	21.44	19.51
Calcium	0.950	0.850	0.800
Avail Phosphorus	0.475	0.425	0.400
Sodium	0.200	0.190	0.185
Crude Fat	4.892	4.557	4.130
Digestible Lysine	1.200	1.080	0.980
Digestible Methionine	0.614	0.552	0.494
Digestible TSAA	0.912	0.832	0.755
Digestible Tryptophan	0.256	0.227	0.199
Digestible Threonine	0.792	0.724	0.666
Digestible Isoleucine	0.879	0.783	0.691
Digestible Valine	0.912	0.832	0.755
Digestible Cystine	0.298	0.279	0.261
Digestible Arginine	1.466	1.306	1.153

[1] Optiphos® – Huvepharma Inc. – Peachtree City, GA

[2] Active drug ingredient salinomycin sodium, 60 g/lb of activity Huvepharma Inc. – Peachtree City, GA (60 g/ton inclusion).). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

[3] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

[4] Vitamin premix added at this rate yields 7,700 IU vitamin A, 5,500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

Experimental Diets. Feeding of the 8 treatment diets began on d 35 post re-randomization. All diets were derived from a common basal as to eliminate nutritional variation between dietary treatments (Table 5-3). The first 7 diets were formulated to determine the dSAA requirement. Cystine remained static in the diet at 0.243% so SAA levels were adjusted by the addition of Met alone. Treatment 1 was the basal diet and contained no additional Met having a digestible methionine (dMet) level of 0.248% and a dSAA level of 0.491%. Incremental increases of DL-Met were included into the diets to reach the summit at treatment 7 with a Met inclusion rate of 0.395% for a 0.639% dMet level and a dSAA level of 0.882%. Treatment 4 was considered to be the breakpoint with a Met inclusion rate of 0.197% for a dMet level of 0.444% and a dSAA level of 0.687%. Treatment 8 included HMTBa in place of DL-Met. Treatment 8 included HMTBa at the same dMet and dSAA level as treatment 4 to determine the bioavailability of HMTBa. All diets contained the same level of ingredients with the exception of Met and corn starch, which was adjusted accordingly. Diets were calculated to contain 0.90% dLys (1.03% total Lys) (tLys), and were formulated on an ideal AA basis.

Table 5-3. Experimental diets and calculated nutrient content for male broilers fed diets with varying levels of methionine.

Ingredient Profile	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 7	Trt 8
CORN	59.539	59.539	59.539	59.539	59.539	59.539	59.539	59.539
Wheat	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Soybean Meal (48% CP)	22.228	22.228	22.228	22.228	22.228	22.228	22.228	22.228
Meat & Bone Meal	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500
Limestone	0.654	0.654	0.654	0.654	0.654	0.654	0.654	0.654
Monocalcium Phosphate	0.960	0.960	0.960	0.960	0.960	0.960	0.960	0.960
Copper Sulfate Pentahydrate	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Sodium Chloride	0.273	0.273	0.273	0.273	0.273	0.273	0.273	0.273
Sodium Bicarbinate	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
L-Lysine HCl, 78.8%	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204
DL-Methionine, 99%	-	0.066	0.132	0.197	0.263	0.329	0.395	-
HMTBa[1], 88%	-	-	-	-	-	-	-	0.222
L-Threonine, 98.0%	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.088
Choline 60%	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.162
Fat-A/V Blend	2.614	2.614	2.614	2.614	2.614	2.614	2.614	2.614
Corn Starch	0.395	0.329	0.263	0.197	0.132	0.066	-	0.173
Trace Mineral Premix[3]	0.059	0.059	0.059	0.059	0.059	0.059	0.059	0.059
Vitamin Premix[4]	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Calculated Nutrient Content %								
AME (kcal/kg)	3,190	3,190	3,190	3,190	3,190	3,190	3,190	3,190
Crude Protein (%)	18.146	18.146	18.146	18.146	18.146	18.146	18.146	18.146
Calcium	0.750	0.750	0.750	0.750	0.750	0.750	0.750	0.750
Avail Phosphorus	0.375	0.375	0.375	0.375	0.375	0.375	0.375	0.375
Sodium	0.180	0.180	0.180	0.180	0.180	0.180	0.180	0.180
Crude Fat	5.328	5.328	5.328	5.328	5.328	5.328	5.328	5.328
Digestible Lysine	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900
Digestible Methionine	0.248	0.313	0.379	0.444	0.509	0.574	0.639	0.444
Digestible TSAA	0.491	0.556	0.622	0.687	0.752	0.817	0.882	0.687
Digestible Tryptophan	0.187	0.187	0.187	0.187	0.187	0.187	0.187	0.187
Digestible Threonine	0.621	0.621	0.621	0.621	0.621	0.621	0.621	0.621
Digestible Isoleucine	0.643	0.643	0.643	0.643	0.643	0.643	0.643	0.643
Digestible Valine	0.702	0.702	0.702	0.702	0.702	0.702	0.702	0.702
Digestible Cystine	0.243	0.243	0.243	0.243	0.243	0.243	0.243	0.243
Digestible Arginine	1.062	1.062	1.062	1.062	1.062	1.062	1.062	1.062
Analyzed Nutrient Content %								
Crude Protein	18.51	18.51	18.51	18.51	18.51	18.51	18.51	18.51
Digestible Lysine	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900
Digestible Methionine	0.282	0.343	0.405	0.466	0.527	0.588	0.650	-
Digestible TSAA	0.525	0.586	0.648	0.709	0.770	0.831	0.893	-
Digestible Threonine	0.750	0.750	0.750	0.750	0.750	0.750	0.750	0.750

Table 5-3 Continued.

Ingredient Profile	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 7	Trt 8
Digestible Arginine	1.180	1.180	1.180	1.180	1.180	1.180	1.180	1.180
Digestible Isoleucine	0.770	0.770	0.770	0.770	0.770	0.770	0.770	0.770
Digestible Leucine	1.490	1.490	1.490	1.490	1.490	1.490	1.490	1.490
Digestible Valine	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900
Digestible Tryptophan	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.240

[1] Rhodimet AT88, Adisseo USA, Alpharetta, GA

[2] Active drug ingredient salinomycin sodium, 60 g/lb of activity Huvepharma Inc. – Peachtree City, GA (60 g/ton inclusion). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

[3] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

[4] Vitamin premix added at this rate yields 7,700 IU vitamin A, 5,500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

Statistical Analysis. On d 35 for re-randomization, data were analyzed using PROC MIXED procedure of SAS (2004) included block as a random effect. Digestible SAA requirements were determined using a quadratic broken-line model (QB) using ProcNLIN. Contrasts were analyzed using a standardized T-test.

Results

Body Weight and Body Weight Gain. Broiler performance responded in a curvilinear relationship to DLM with the exception of FC. Broken-line analysis for average male BW at d 49 exhibited, when fed increasing levels of DLM, increased BW with maximum performance achieved at a dSAA requirement of 0.813% (Table 5-4) (Figure 5-1). When comparing the breakpoint dSAA level of 0.687% for both the DLM and HMTBa, no differences were observed between the Met sources.

Table 5-4. Quadratic broken-line analysis for average body weight, body weight gain, mortality adjusted feed conversion ratio (FCR), and cumulative feed conversion ratio (cFCR) of male broilers fed a diet with varying levels of methionine inclusion.

	Body Weight	**Body Weight Gain**	**FCR**	**cFCR**
R^2	71.06	70.61	97.4	97.7
dSAA Requirement, %	0.813	0.823	0.772	0.779
dSAA:dLys Ratio, %	90.3	91.4	85.8	86.6
Trt 4 vs. 8 (p-value)	0.407	0.378	0.934	0.901

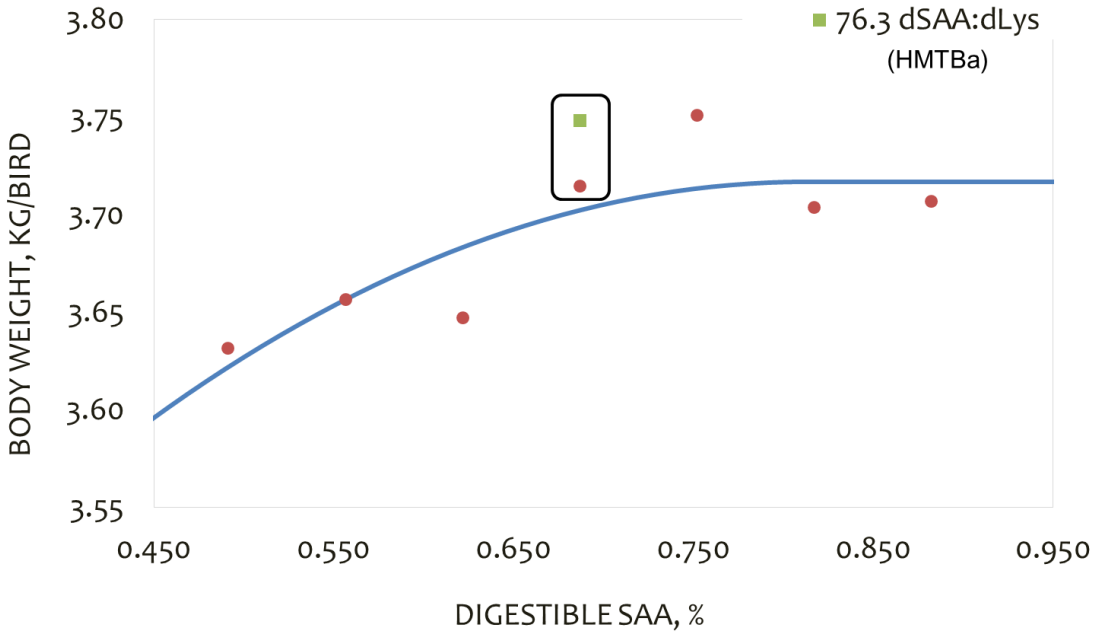

Figure 5-1. Quadratic broken-line analysis for average body weight of male broilers from 35 to 49 days-of-age; R^2=71.06, Max Response=3.72, dSAA Requirement=0.813%, dSAA:dLys Ratio=90.3%, and TRT 4 vs. 8 (p-value)=0.407.

For d 35 to 49 BWG, observations for peak performance were slightly higher at a dSAA requirement of 0.823% when compared to dSAA requirement for BW (Table 5-4) (Figure 5-2). When comparing the DLM and the HMTBa, differences were not observed regarding BWG.

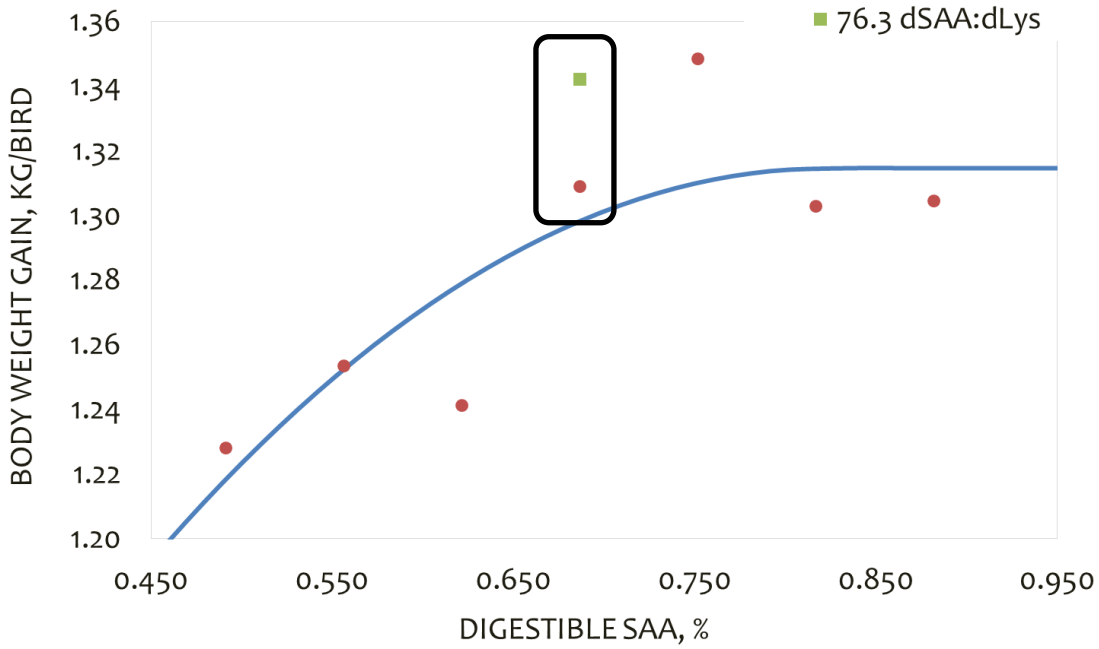

Figure 5-2. Quadratic broken-line analysis for body weight gain of male broilers from 35 to 49 days-of-age; R^2=70.61, Max Response=1.32, dSAA Requirement=0.823%, dSAA:dLys Ratio=91.4%, and TRT 4 vs. 8 (p-value)=0.378.

Mortality Feed Conversion Ratio and Cumulative FCR. Mortality corrected FCR for d 35 to 49 resulted in a reduced FCR as DLM increased in the diet with the ideal dSAA level at 0.772% (Table 5-4) (Figure 5-3). When comparing the DLM source to the HMTBa, observed results for mortality adjusted FCR were similar.

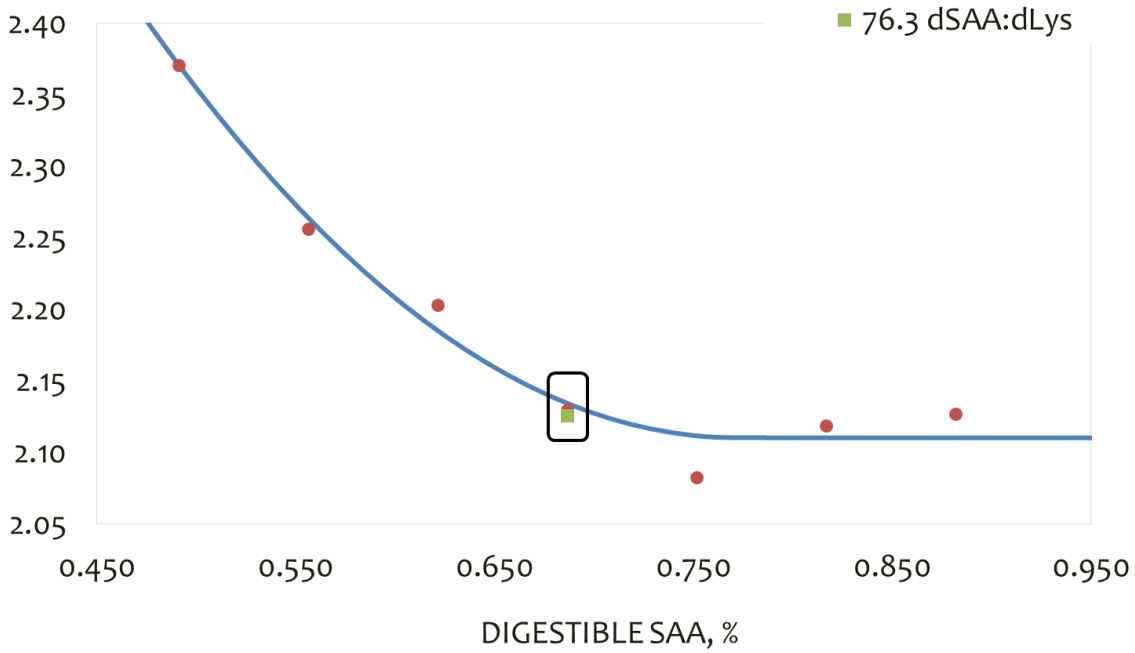

Figure 5-3. Quadratic broken-line analysis for FCR of male broilers from 35 to 49 days-of-age; R^2=97.4, Max Response=2.11, dSAA Requirement=0.772%, dSAA:dLys Ratio=85.8%, and TRT 4 vs. 8 (p-value)=0.934.

Cumulative feed conversion (d 1 to 49) was calculated treating all birds equally to d 35 and then including the experimental phase. There is a similar trend to cumulative FCR when compared to the experimental phase FCR with the dSAA requirement being 0.779% (Table 5-4) (Figure 5-4). Again, when comparing the powder methionine source to the liquid methionine source, no differences were observed in the products for cFCR.

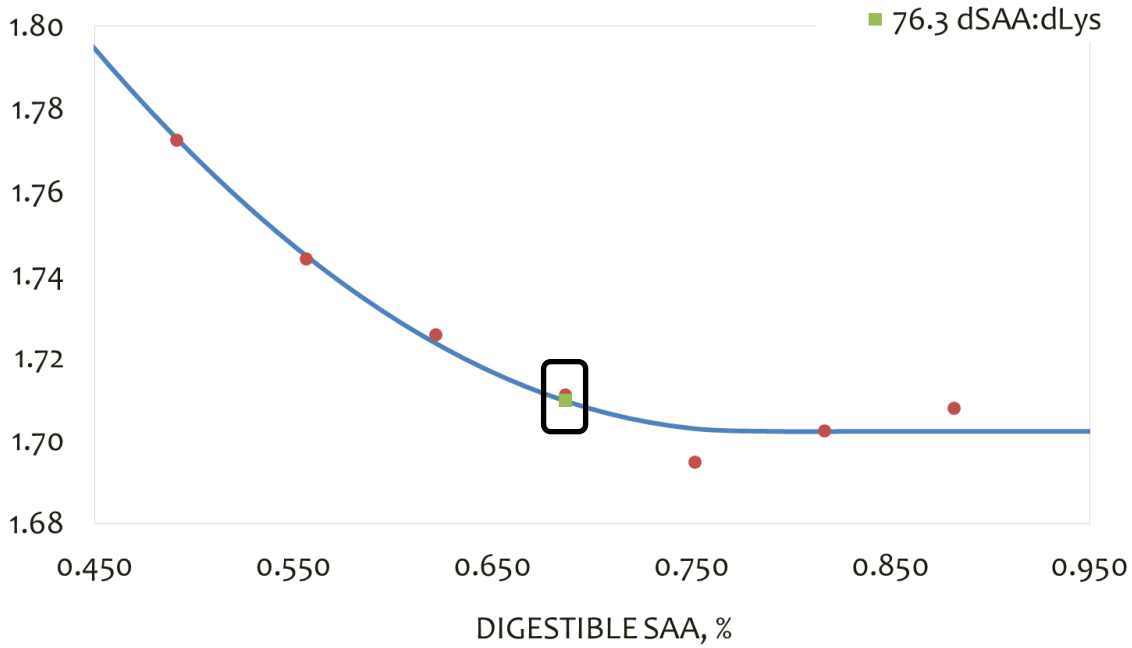

Figure 5-4. Quadratic broken-line analysis for cFCR of male broilers from 0 to 49 days-of-age; R^2=97.7, Max Response=1.702, dSAA Requirement=0.779%, dSAA:dLys Ratio=86.6%, and TRT 4 vs. 8 (p-value)=0.901.

DL-Methionine vs. HMTBa. When including DLM and HMTBa at the same dSAA level, no differences were observed between the two Met sources regardless of the parameter evaluated (Table 5-4).

Discussion

Broiler growth rates and meat yields have increased significantly over the last several years largely due to genetic selection. The enhancement in growth rates and meat yields plays a critical role in diet formulation, particularly with AA inclusion. Amino acids are crucial for broiler performance and development. There are numerous synthetic AA products available for diet formulation to ensure the broiler is able to meet its maintenance requirement. The two supplemental Met sources that are commonly

used are DLM and HMTBa (Zhang and Guo, 2008). The majority of research for AA inclusion is focused on early development (0 to 28 days) or is in need of revision. Information is needed on the dSAA requirement of the current broiler during later phases of grow-out. The goal for the current trial is to provide a dSAA requirement with current broiler genetics from d 35 to 49, while determining the bioavailability of HMTBa.

Previous research has shown that Met requirement may be influenced by many factors such as rearing conditions, genetics, sex, crude protein levels, and addition of drugs or other feed ingredients such as copper and choline (Petel et al., 1980; Jensen et al., 1989; Garcia and Batal, 2005; Dilger et al., 2007; Lumpkins et al., 2007; Karimi et al., 2011). In the current experiment, the requirement for dSAA was higher than the assumed breakpoint of 0.687%. Choline was included in the diet at 0.162%, which is a sufficient amount so not to interfere with the methyl donation from Met. Copper is commonly included into broiler diets due to its antibacterial or bacteriostatic properties to improve broiler performance (Ewing et al., 1998; Pesti and Bakalli, 1998; Arias and Koutsos, 2006; Karimi et al., 2011) and serve as an alternative to antibiotic growth promoters (AGPs). Although studies have shown that increased copper inclusion can improve broiler performance, higher levels of copper inclusion have had variable results (Jensen et al., 1989; Banks et al., 2004; Arias and Koutsos, 2006; Pang and Applegate, 2007), with studies using copper sulfate inclusion levels in excess of 300 ppm resulting in deleterious effects on broiler performance (Persia et al., 2004; Luo et al., 2005). Jensen and co-workers (1989) reported variable results over 2 experiments with one

resulting in a significant interaction between copper (240 ppm) and Met, with larger improvements in feed efficiency with Met supplementation in the presence of copper. However, observations for the second trial resulted in greater improvements in BWG in males in the absence of copper. Karimi and colleagues (2011) reported that including copper at 125 ppm improved FCR during the starter period when compared to no supplemented copper. However, supplementing copper at 250 ppm, resulted in adverse effects on BW, BWG, and feed intake. Copper sulfate pentahydrate was included in the diet for the current trial at 200 ppm. We cannot conclude if the presence of copper impacted our results.

Past research focused on the minimum requirements of dSAA throughout the literature may have become inaccurate as genetic selection for larger, faster, and higher yielding birds continues. This continued selection for increased growth rate and yield will require changes in diet formulation to meet the demands for nutrients to support growth rate. In the late 1980's, Jensen and co-workers (1989) recommended that the minimum dSAA level for 3 to 6 weeks be 0.78%, which was higher than the minimum recommended in literature (0.72%) during that era. Similar results were observed in a series of experiments conducted by Lumpkins and colleagues (2007) in which the authors reported observations from d 21- 42 indicated that dSAA requirement for maximal feed efficiency (0.64%) was 10% higher than for maximal BWG (0.55%). This report is in contrast with the data from current trial which observed a higher dSAA requirement for BWG (0.82%) as compared to FCR (0.77%). However, these two studies varied considerably in the age of the broiler as Lumpkins and colleagues (2007)

focused on a 3 week period ending on d 42 and this project initiated the study on d 35 for a 2 week evaluation period. The results of the current study suggest that dSAA needs for maximal BWG exceed those for peak feed efficiency.

Feed consumption was not influenced in the current trial with increasing levels of Met supplementation. These results contradict findings reported by Lin and Shih (2000), Carew, Mcmurtry, and Alster (2003), and Attia, Hassan, Shehatta, and El-Hady (2005) which suggest that broilers increase consumption in order to compensate for a Met deficiency, however these reports are more than a decade old. Other researchers (Kiraz and Sengul, 2005; Bunchasak, 2009) have reported that AA or Met deficiency can reduce appetite. More recent publications by Zhang and Guo (2008) and Goulart and co-workers (2011) reported that feed consumption was not influenced by Met supplementation which is in agreement with the current experiment. Goulart and co-workers (2011) reported that the greatest performance was achieved during the finisher phase (36 to 42d) at a dSAA level of 0.661% which is lower than observations in the current trail, regardless of the parameter of focus. In the present study, peak BWG was achieved at a dSAA requirement of 0.823% with significant improvements in BW and mortality adjusted FCR with increasing supplemental Met. In contrast, Conde-Aguilera and co-workers (2013) reported no differences in performance parameters of broilers at 42 days-of-age when feeding a diet deficient in SAA compared to feeding a diet sufficient in SAA.

When comparing DLM and HMTBa, observations resulted in both products being similar in all parameters evaluated. Previous research has also concluded that both

Met sources can be absorbed at equal rates (Knight and Dibner, 1984) and that the compounds are considered equal on an equimolar basis (Daenner and Bessei., 2003; Motl et al., 2005; Zou et al., 2015). Other researchers have reported obtaining maximum performance when using DLM as compared to HMTBa (Sauer et al., 2008; Vedenov and Pesti, 2010). Vazquez-Anon and colleagues (2006) stated that HMTBa was able to outperform DLM at high concentrations of dSAA when comparing gain-response curves to dietary concentrations of the Met sources. However, at lower dSAA concentrations, broilers fed DLM resulted in greater BWG when compared to broilers fed HMTBa. Zou and colleagues (2015) suggested that HMTBa can outperform DLM by decreasing the amount of feed necessary when supplementing Met at higher levels although this was not measured in the current experiment. Results from the current experiment suggest that broilers are able to utilize both DLM and HMTBa equally, and the bioavailability of HMTBa is 100%.

CHAPTER VI

IMPACT OF VARIABLE CORN NUTRIENT CONTENT, AME PREDICTION, AND XYLANASE INCLUSION ON GROWTH PERFORMANCE[*]

Introduction

Corn is a primary source of energy in standard U.S. broiler diets. Understanding the factors which influence the energy value of corn is critical for capitalizing on the nutritive value of the grain. The corn endosperm, which is a starch-protein matrix, is crucial for influence on the nutritional value of the grain. There are two types of endosperm; floury and vitreous. The floury and vitreous textures are a result of differences in protein and starch interactions (Rooney and Pflugfelder, 1986; Cowieson, 2005; Gayral et al., 2015). Floury endosperm is more open or loose with less encapsulation by proteins compared to the vitreous endosperm which is hard and tightly compacted (Rooney and Pflugfelder, 1986; Cowieson, 2005; Gayral et al., 2015). Increased vitreousness is negatively related to the digestibility of starch. Starch is contained in the corn endosperm, thus understanding the biochemistry of the endosperm would aid in improving starch utilization (Rooney and Pflugfelder, 1986). A challenge with starch digestion in corn is due to starch being embedded in the protein matrix leading to increased protein-starch interactions (Rooney and Pflugfelder, 1986; Cowieson, 2005; Gayral et al., 2015). There are numerous factors that impact corn

[*] Reprinted with the permission from "Impact of variable corn nutrient content, AME prediction, and xylanase inclusion on growth performance" by R. E. Latham, M. P. Williams, C. Flores, H. V. Masey O'Neill, T. W. York, and J. T. Lee, 2016. Journal of Applied Poultry Research, 00, 1-14, Copyright 2016 by Oxford University Press and Copyright Clearance Center.

starch digestibility such as plant species, physical composition, inhibitors, structure of starch lipid/protein/starch matrices, and the existence of anti-nutritive factors (Leigh, 1994; Brown, 1996; Cromwell et al., 1998; Collins and Moran, 2001; Gehring et al., 2012). Corn nutrient value also varies in terms of composition and variety from year to year. Geographical location has an effect on corn nutrient digestibility attributable to agronomic conditions, post-harvest processing, and storage conditions (Gehring et al., 2012). Cowieson (2005) reported that differences in corn samples can yield variability in AME of more than 400 kcal/kg. Metabolizable energy (ME) is the standard measure of energy availability in poultry. Therefore having a rapid and accurate AME value of corn, as it is a main contributor of AME in standard U.S. poultry diets, would be ideal.

Near infrared reflectance (NIR), which has gained credibility over the years, is now commonly used for the prediction of proximate analysis and amino acid (AA) content on a number of ingredients. The primary attractiveness of NIR relates to rapid results and relatively low cost, with availability of calibrations for crude protein, fat, fiber, ash, moisture, and many other nutrients. These calibrations are derived from a large database of ingredient profiles from different geographical locations. Although determination of AME value for corn with NIR is a greater challenge, there are several calibrations which are available for the prediction of corn AME available. The calibration in this study predicts the AME using parameters such as proximate analysis vitreousness, and protein solubility index (PSI)[8,9]. Two previous trials were conducted

[8] AB VISTA, Corn Quality Service, Marlborough, UK
[9] Aunir, Near Infrared Calibration, Towcester, UK

evaluating corn from 6 geographical locations (Masey O'Neill et al., 2013, 2014). The results of these studies indicated a linear relationship between the predicted AME value of corn using the NIR calibration and the observed mortality corrected FCR indicating an accurate prediction of corn AME value associated with broiler performance. Using this prediction equation reflects more accurate nutrient content prior to diet formulation allowing for more efficient diet formulation.

Supplementation of diets with endogenous enzymes such as carbohydrases is a common practice used in the broiler industry to aid in increasing nutrient utilization of lower quality ingredients. Supplementation of enzymes has predominantly been more effective in high fiber based diets (Bedford and Classen, 1992; Bedford and Morgan, 1996; Choct, 2006). In the U.S., the majority of diets are corn-SBM based, which have had variable results with exogenous enzyme supplementation (Bedford and Morgan, 1996; Meng and Slominski, 2005; Choct, 2006). Corn is a main contributor of AME at approximately 65% in common broiler starter diets (Cowieson, 2005). Carbohydrases such as xylanase have been incorporated into broiler diets, making dietary nutrients more available, and have shown to significantly improve broiler performance in reduced energy diets (Bedford and Classen, 1993; Wang et al., 2005; Esmaeilipour et al., 2011; Coppedge et al., 2012; Masey O'Neill et al., 2012; Williams et al., 2014). Xylanase response in corn-SBM diets has been inconsistent between studies (Bedford and Morgan, 1996; Meng and Slominski, 2005; Choct, 2006), which may be related to variable corn quality. If an accurate corn AME value could be predicted using NIR, potentially xylanase response may also be predicted. The objective of the current

experiment (Experiment 4) was to compare broiler performance when diets were formulated using the predicted AME values, based on NIR calibrations, for corns from different regions to diets formulated using the NRC (1994) AME value for corn, with and without xylanase.

Materials and Methods

Experimental Design. The impact of varying corn AME on broiler performance using an NIR calibration to predict corn AME in the absence or presence of xylanase on broiler growth performance was evaluated in a completely randomized block design with 3 dietary phases during a 42 d growth experiment. The experimental design consisted of a 3 (corn source) x 2 (formulation; adjusted vs non-adjusted value) x 2 (xylanase inclusion, 0 vs 16,000 BXU/kg) factorial yielding a total of 12 treatments (Table 6-1). All non-adjusted diets were formulated using the NRC (1994) value for corn.

Table 6-1. Experimental treatments for the 3x2x2 factorial.

Corn Source	**Xylanase[1]**	**Adjustment Factor**
A	Enzyme	Non-adjusted[2]
A	Control	Non-adjusted
A	Enzyme	Adjusted[3]
A	Control	Adjusted
B	Enzyme	Non-adjusted
B	Control	Non-adjusted
B	Enzyme	Adjusted
B	Control	Adjusted
C	Enzyme	Non-adjusted
C	Control	Non-adjusted
C	Enzyme	Adjusted
C	Control	Adjusted

[1] ECONASE XT, AB VISTA, Marlborough, UK. Xylanase was included in the diets at 16,000 BXU/kg at an inclusion rate of 100 g/ton

[2] Non-adjusted diets were formulated using corn AME values from the NRC (1994). All corn sources were considered to have equal AME values, included at the same percentage, and formulated to be isonitrogenous and isocaloric

[3] Adjusted diets were formulated using the predicted corn AME values determined using an NIR and a calibration for corn AME developed by Aunir and AB Vista. The corns were included into the diets at different percentages and formulated to be isonitrogenous and isocaloric

On d of hatch, 2,400 male Cobb 500 broiler chicks were randomly allotted to floor-pens and dietary treatments based on initial body weight. The study consisted of a total of 120 pens with 10 blocks of 12 pens, each containing 20 chicks at d of age. The pens were 3.34 m^2 floor pens with nipple drinkers and a tube feeder. Feed and water were available *ad libitum.* Chicks were provided age appropriate supplemental heat and were subject to an industry type lighting program consisting of; d 1 to 8, 24 h of light at 2 foot candles, d 9 to 18, 16 h of light at 0.75 foot candle, d 19 to 32, 18 h of light at 0.1

foot candle, and d 33 to 42, 20 h of light at 0.05 foot candle. Broilers were reared on used litter from 2 previous flocks that was top dressed with fresh pine shavings. All broilers and feed were weighed by pen on d 18, 33, and 42 to determine average BW, mortality adjusted FCR, FC, and body weight adjusted FCR (waFCR). Body weight adjusted FCR was calculated using 27g of d 42 BW being equal to 1 point of feed conversion. Other evaluated parameters included cFCR, cFC, and mortality. All animal husbandry procedures were conducted in accordance with an approved animal use protocol (IACUC).

Corn Analysis. A total of 5 corn sources (A, B, C, D, and E; Table 6-2) were obtained initially and selected based on the predicted AME using the NIR calibration[2] and the calibrations were based on wet chemistry analyses of 1,000 corn samples, as described by Piotrowski and colleagues (2011). The intent was to obtain 3 corn sources with a wide variation in energy based on having obtained and analyzed corn from these same regions in 2 previous experiments. The corn sources were sampled prior to the beginning of the study for proximate analysis. After reviewing the nutrient and AME values, 3 corns were selected (A, B and C). There was approximately a 105 kcal/kg spread from the highest (A) to the lowest (C) corn AME prediction value. Proximate and physiochemical analyses were conducted using NIR spectroscopy with a Foss 6500 NIR spectrophotometer[10].

[10] Foss NIR Systems Inc., Maryland, USA

Table 6-2. Near infrared reflectance predicted apparent metabolizable energy[1, 2] and analyzed composition (% dry matter) of corn associated with different geographical locations.

Corn Source	Predicted AME (as is)	Starch	Protein	Oil	Fiber	Moisture	PSI	Vitreousness
A	3323	76.95	8.01	3.67	2.60	12.42	43.61	55.06
B	3237	76.89	8.12	3.78	2.67	14.36	41.39	57.18
C	3218	77.99	7.74	3.82	2.58	16.34	46.50	56.09
D	3297	76.63	8.20	3.74	2.64	13.00	44.54	57.23
E	3232	76.46	8.21	3.93	2.69	14.64	45.01	59.99

[1] AB VISTA, Corn Quality Service, Marlborough, UK
[2] Aunir, Near Infrared Calibration, Towcester, UK

Experimental Diets. Diets were corn-SBM based and formulated to be iso-nitrogenous and iso-energetic (Table 6-3, 6-4, and 6-5) within each phase. Digestible AA and available phosphorus levels were derived from current industry practices. The non-adjusted diet was formulated based on the NRC (1994) AME values for corn with fat inclusion at 1.5% in for the starter phase. As expected, fat inclusion varied based on the predicted AME value of corn for the adjusted diets. The predicted AME of the corn sources was lower than the NRC (1994) AME values. As a result, additional fat was included into the diets. Xylanase[11] was included to the diets at 0 or 16,000 BXU/kg. Pelleting temperatures were maintained between 74˚ and 76˚ C. The starter diet was fed as a crumble with the remaining phases fed as a pellet. A starter diet was fed from d 0 to 18, grower from d 19 to 33, and finisher from d 34 to 42. Crude protein was determined

[11] ECONASE XT, AB VISTA, Marlborough, UK

using AOAC by combustion (AOAC 990.03) and an ether extraction to determine crude fat (AOAC 920.39).

Table 6-3. Experimental diets and calculated nutrient content for male broilers fed a diet with varying corn sources, with a NRC (1994) or predicted AME value of corn, and the addition of enzyme or control during the starter phase.

Ingredient Profile	Starter %			
	Non-Adj	Adjusted		
	NRC	A	B	C
Corn	61.14	59.82	58.76	57.83
Soybean Meal (48% CP)	32.86	33.74	33.78	34.41
Fat – A/V Blend	1.56	2.04	3.05	3.36
Limestone	1.58	1.57	1.57	1.57
DL - methionine	0.25	0.26	0.26	0.26
Vitamin premix[1]	0.25	0.25	0.25	0.25
L-lysine HCl	0.22	0.20	0.20	0.18
Sodium Chloride	0.45	0.46	0.46	0.46
Sodium Bicarbonate	0.01	N/A	N/A	N/A
L-Threonine	0.01	0.01	0.01	0.01
Trace Mineral Px[2]	0.05	0.05	0.05	0.05
Mono Calcium Phosphate	1.57	1.56	1.57	1.56
Coban 90[3]	0.05	0.05	0.05	0.05
Calculated Nutrient Content %				
AME (kcal/kg)	3000	3000	3000	3000
Crude Protein (%)	21.10	21.10	21.10	21.10
Calcium	0.95	0.95	0.95	0.95
Avail Phosphorus	0.45	0.45	0.45	0.45
Sodium	0.20	0.20	0.20	0.20
Crude Fat	4.16	4.56	5.59	5.89
Digestible Lysine	1.30	1.30	1.30	1.30
Digestible Methionine	0.58	0.58	0.58	0.58
Digestible TSAA	0.93	0.93	0.93	0.93
Digestible Tryptophan	0.25	0.25	0.26	0.26
Digestible Threonine	0.81	0.81	0.81	0.81
Digestible Isoleucine	0.85	1.31	1.30	1.31
Digestible Valine	0.95	0.99	0.99	1.00
Digestible Cystine	0.34	0.35	0.35	0.35
Digestible Arginine	1.37	1.42	1.42	1.43

Table 6-3 Continued.

Analyzed Nutrient Content %				
Crude Protein	21.13	22.90	22.70	19.70
Crude Fat	3.98	4.52	4.61	5.99

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

[3] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

[4] Expected xylanase activity was 16,000 BXU/kg with xylanase recovery at 15,100 BXU/kg for corn A non-adjusted; 18,200 BXU/kg for corn A adjusted; 17,600 BXU/kg for corn B non-adjusted; 15,700 BXU/kg for corn B adjusted; 17,600 for corn C non-adjusted; and 13,300 BXU/kg for corn C adjusted

Table 6-4. Experimental diets and calculated nutrient content for male broilers fed a diet with varying corn sources, with a NRC (1994) or predicted AME value of corn, and the addition of enzyme or control during the grower phase.

Ingredient Profile	Grower %			
	Non-Adj	Adjusted		
	NRC	A	B	C
Corn	63.47	61.96	60.82	59.92
Soybean Meal (48% CP)	29.62	30.67	30.72	31.36
Fat – A/V Blend	2.94	3.45	4.52	4.82
Limestone	1.36	1.36	1.39	1.36
DL - methionine	0.24	0.24	0.24	0.24
Vitamin premix[1]	0.25	0.25	0.25	0.25
L-lysine HCl	0.21	0.18	0.18	0.17
Sodium Chloride	0.37	0.40	0.40	0.42
Sodium Bicarbonate	0.13	0.08	0.09	0.06
L-Threonine	0.01	0.01	0.01	0.01
Trace Mineral Px[2]	0.05	0.05	0.05	0.05
Mono Calcium Phosphate	1.31	1.30	1.30	1.30
Coban 90[3]	0.05	0.05	0.05	0.05
Calculated Nutrient Content %				
AME (Kcal/kg)	3113	3113	3113	3113
Crude Protein (%)	20.10	20.10	20.10	20.10
Calcium	0.82	0.82	0.83	0.82
Avail Phosphorus	0.39	0.39	0.39	0.39
Sodium	0.20	0.20	0.20	0.20
Crude Fat	5.63	6.01	7.09	7.39
Digestible Lysine	1.20	1.20	1.20	1.20
Digestible Methionine	0.54	0.54	0.54	0.54
Digestible TSAA	0.88	0.88	0.88	0.88
Digestible Tryptophan	0.23	0.24	0.24	0.24
Digestible Threonine	0.75	0.75	0.75	0.75
Digestible Isoleucine	0.81	1.27	1.26	1.26
Digestible Valine	0.91	0.93	0.92	0.93
Digestible Cystine	0.33	0.33	0.33	0.33
Digestible Arginine	1.30	1.32	1.32	1.33
Analyzed Nutrient Content %				
Crude Protein	20.00	20.20	20.80	20.10
Crude Fat	5.46	5.56	6.50	7.21

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

[3] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

[4] Expected xylanase activity was 16,000 BXU/kg with xylanase recovery at 18,300 BXU/kg for corn A non-adjusted; 20,700 BXU/kg for corn A adjusted; 18,300 BXU/kg for corn B non-adjusted; 20,300 BXU/kg for corn B adjusted; 18,800 for corn C non-adjusted; and 19,500 BXU/kg for corn C adjusted

Table 6-5. Experimental diets and calculated nutrient content for male broilers fed a diet with varying corn sources, with a NRC (1994) or predicted AME value of corn, and the addition of enzyme or control during the finisher phase.

Ingredient Profile	Finisher %			
	Non-Adj	Adjusted		
	NRC	A	B	C
Corn	67.54	66.10	64.94	63.02
Soybean Meal (48% CP)	25.46	26.41	26.46	28.00
Fat – A/V Blend	3.17	3.70	4.81	5.28
Limestone	1.24	1.24	1.24	1.23
DL - methionine	0.22	0.23	0.23	0.22
Vitamin premix[1]	0.25	0.25	0.25	0.25
L-lysine HCl	0.23	0.21	0.21	0.16
Sodium Chloride	0.21	0.24	0.23	0.29
Sodium Bicarbonate	0.28	0.24	0.25	0.18
L-Threonine	0.01	0.01	0.01	N/A
Trace Mineral Px[2]	0.05	0.05	0.05	0.05
Mono Calcium Phosphate	1.29	1.28	1.29	1.28
Coban 90[3]	0.05	0.05	0.05	0.05
Calculated Nutrient Content %				
AME (kcal/kg)	3168	3168	3168	3168
Crude Protein (%)	18.44	18.44	18.44	18.44
Calcium	0.76	0.76	0.76	0.76
Avail Phosphorus	0.38	0.38	0.38	0.38
Sodium	0.18	0.18	0.18	0.18
Crude Fat	5.97	6.36	7.50	7.93
Digestible Lysine	1.10	1.10	1.10	1.10
Digestible Methionine	0.51	0.51	0.51	0.51
Digestible TSAA	0.82	0.82	0.82	0.82
Digestible Tryptophan	0.23	0.24	0.24	0.25
Digestible Threonine	0.69	0.69	0.69	0.69
Digestible Isoleucine	0.74	1.22	1.21	1.22
Digestible Valine	0.84	0.85	0.85	0.87
Digestible Cystine	0.30	0.31	0.31	0.31
Digestible Arginine	1.15	1.17	1.17	1.20
Analyzed Nutrient Content %				
Crude Protein	18.27	19.5	19.5	18.7
Crude Fat	5.92	5.67	6.82	7.22

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*

[4] Expected xylanase activity was 16,000 BXU/kg with xylanase recovery at 20,700 BXU/kg for corn A non-adjusted; 18,000 BXU/kg for corn A adjusted; 19,000 BXU/kg for corn B non-adjusted; 20,700 BXU/kg for corn B adjusted; 16,900 for corn C non-adjusted; and 20,000 BXU/kg for corn C adjusted

Statistical Analysis. All phase and cumulative data were subject to a 3x2x2 factorial ANOVA using the General Linear Model (JMP Pro 11). Multiple significant two-way interactions were observed, thus 2-way data were analyzed via a two factor factorial to assist with interpretation of the data. Main effect and treatment means were deemed significantly different at $p \leq 0.05$ and were further separated by LSMEANS Test. The 3-way model is presented in Table 6-6; Table 6-7 presents the 2-way model for corn source and adjustment; Table 6-8 presents the 2-way model enzyme inclusion and adjustment factor; Table 6-9 presents the 2-way model for corn source and enzyme inclusion. No significant 3-way interaction (corn source*enzyme inclusion*adjustment factor) were observed in the data set throughout the trial. To reduce the amount of tables, only BW and cumulative data will be presented.

Table 6-6. Average body weight, cumulative mortality adjusted feed conversion ratio (cFCR), cumulative feed consumption (cFC), and cumulative weight adjusted feed conversion ratio (waFCR) of male broilers fed a diet with varying corn sources, with a NRC (1994) or predicted AME value of corn, and the addition of enzyme or control (3-way interaction).

			Body Weight			cFCR	cFC	waFCR
Corn Source	**Xylanase**	**Adjustment Factor**	**Day 18 (g)**	**Day 33 (kg)**	**Day 42 (kg)**	**Day 0-42**	**Day 0-42 (g)**	**Day 0-42**
A	Enzyme	Non-adjusted	623.8	2.059	2.975	1.615	110.9	1.588
A	Control	Non-adjusted	625.5	2.038	2.969	1.606	110.0	1.581
A	Enzyme	Adjusted	656.9	2.069	2.987	1.602	110.9	1.571
A	Control	Adjusted	638.0	2.066	3.012	1.599	110.0	1.557
B	Enzyme	Non-adjusted	624.8	1.994	2.902	1.639	110.9	1.640
B	Control	Non-adjusted	605.8	1.983	2.860	1.647	110.9	1.662
B	Enzyme	Adjusted	649.8	2.054	2.982	1.584	110.9	1.553
B	Control	Adjusted	636.5	2.065	2.974	1.595	110.0	1.567
C	Enzyme	Non-adjusted	608.3	1.980	2.912	1.651	111.8	1.646
C	Control	Non-adjusted	590.4	1.922	2.804	1.670	110.0	1.706
C	Enzyme	Adjusted	642.4	2.075	2.989	1.615	112.7	1.584
C	Control	Adjusted	629.9	2.065	2.993	1.591	111.0	1.556
Main Effect Means								
Corn Source								
A			636.1	2.058	2.986	1.605	110.5	1.574
B			629.2	2.024	2.929	1.616	110.7	1.605
C			617.8	2.011	2.924	1.632	111.4	1.623
	Xylanase							
-	Control		621.0	2.023	2.935	1.617	110.3	1.605
-	Enzyme		634.4	2.038	2.958	1.617	111.4	1.597
		Adjustment Factor						
		Non-adjusted	613.1	1.996	2.903	1.637	110.8	1.637
		Adjusted	642.3	2.066	2.989	1.597	110.9	1.565

Table 6-6 Continued.

			Body Weight	cFCR	cFC	waFCR		
Corn Source	**Xylanase**	**Adjustment Factor**	**Day 18 (g)**	**Day 33 (kg)**	**Day 42 (kg)**	**Day 0-42**	**Corn Source**	**Xylanase**
	p-value							
Corn Source			0.001	<0.001	<0.001	0.001	0.309	<0.001
Xylanase			0.001	0.079	0.064	0.941	0.045	0.332
Adjustment			<0.001	<0.001	<0.001	<0.001	0.745	<0.001
Corn x Xylanase			0.678	0.266	0.118	0.483	0.574	0.276
Corn x Adjustment			0.318	<0.001	0.002	0.001	0.524	<0.001
Xylanase x Adjustment			0.680	0.094	0.017	0.275	0.025	0.037
Xylanase x Corn x Adjustment			0.272	0.751	0.299	0.120	0.594	0.069

[1] Weight adjusted FCR was adjusted to an equal BW using 27g of body weight being equal to one point of FCR
[2] ECONASE XT, AB VISTA, Marlborough, UK. Xylanase was included in the diets at 16,000 BXU/kg at an inclusion rate of 100g/ton
[3] Non-adjusted diets were formulated using corn AME values from the NRC (1994). All corn sources were considered to have equal AME values, included at the same percentage, and formulated to be isonitrogenous and isocaloric
[4] Adjusted diets were formulated using the predicted corn AME values determined using an NIR and a calibration for corn AME developed by Aunir and AB Vista. The corns were included into the diets at different percentages and formulated to be isonitrogenous and isocaloric.
[5] Means represent 20 birds per pen, 10 pens per diet

Table 6-7. Average body weight, cumulative mortality adjusted feed conversion ratio (cFCR), cumulative feed consumption (cFC), and cumulative waFCR of male broilers fed a diet with varying corn sources, with a NRC (1994) or predicted AME value of corn, and the addition of enzyme or control (2-way interaction between corn source and adjustment factor).

		Body Weight			cFCR	cFC	waFCR
Corn Source	**Adjustment**	**Day 18 (g)**	**Day 33 (kg)**	**Day 42 (kg)**	**Day 0-42**	**Day 0-42 (g)**	**Day 0-42**
A	Non-adjusted	624.7	2.049[a]	2.972[a]	1.610[b]	110.5	1.585[b]
A	Adjusted	647.5	2.068[a]	3.000[a]	1.600[bc]	110.5	1.564[b]
B	Non-adjusted	615.3	1.988[b]	2.881[b]	1.643[a]	110.9	1.651[a]
B	Adjusted	643.2	2.059[a]	2.978[a]	1.589[c]	110.5	1.560[b]
C	Non-adjusted	599.3	1.951[c]	2.858[b]	1.660[a]	110.9	1.676[a]
C	Adjusted	636.2	2.070[a]	2.991[a]	1.603[bc]	111.9	1.570[b]
Main Effect Means							
Corn Source							
A		636.1[a]	2.058	2.986	1.605	110.5	1.574
B		629.2[a]	2.024	2.929	1.616	110.7	1.605
C		617.8[b]	2.011	2.924	1.632	111.4	1.623
	Adjustment						
-	Non-adjusted	613.1[b]	1.996	2.903	1.638	110.8	1.637
-	Adjusted	642.3[a]	2.066	2.989	1.598	110.9	1.565
	p-value						
Corn Source		0.001	<0.001	<0.001	0.001	0.309	<0.001
Adjustment		<0.001	<0.001	<0.001	<0.001	0.745	<0.001
Corn x Adjustment		0.318	<0.001	0.002	0.001	0.524	<0.001

[a-c] Means within a column with different superscripts differ at $p < 0.05$

[1] Weight adjusted FCR was adjusted to an equal BW using 27g of body weight being equal to one point of FCR

[2] ECONASE XT, AB VISTA, Marlborough, UK. Xylanase was included in the diets at 16,000 BXU/kg at an inclusion rate of 100g/ton

[3] Non-adjusted diets were formulated using corn AME values from the NRC (1994). All corn sources were considered to have equal AME values, included at the same percentage, and formulated to be isonitrogenous and isocaloric

[4] Adjusted diets were formulated using the predicted corn AME values determined using an NIR and a calibration for corn AME developed by Aunir and AB Vista. The corns were included into the diets at different percentages and formulated to be isonitrogenous and isocaloric

[5] Means represent 20 birds per pen, 10 pens per diet

Table 6-8. Average body weight, cumulative mortality adjusted feed conversion ratio (cFCR), cumulative feed consumption (cFC), and cumulative waFCR of male broilers fed a diet with varying corn sources, with a NRC (1994) or predicted AME value of corn, and the addition of enzyme or control (2-way interaction between enzyme inclusion and adjustment factor).

		Body Weight			cFCR	cFC	waFCR
Xylanase	**Adjustment**	**Day 18 (g)**	**Day 33 (kg)**	**Day 42 (kg)**	**Day 0-42**	**Day 0-42 (g)**	**Day 0-42**
Control	Adjusted	634.8	2.065	2.993[a]	1.595	110.3	1.560[c]
Enzyme	Adjusted	649.7	2.066	2.986[a]	1.600	111.5	1.569[c]
Control	Non-adjusted	607.2	1.981	2.878[c]	1.641	110.3	1.650[a]
Enzyme	Non-adjusted	619.0	2.011	2.929[b]	1.635	111.2	1.625[b]
Main Effect Means							
Xylanase							
Control		621.0[b]	2.023	2.935	1.618	110.3[b]	1.605
Enzyme		634.4[a]	2.038	2.958	1.618	111.4[a]	1.597
	Adjustment						
-	Non-adjusted	613.1[b]	1.996[b]	2.903	1.638[a]	110.8	1.637
-	Adjusted	642.3[a]	2.066[a]	2.989	1.598[b]	110.9	1.565
	p-value						
Xylanase		0.001	0.079	0.064	0.941	0.045	0.332
Adjustment		<0.001	<0.001	<0.001	<0.001	0.745	<0.001
Xylanase x Adjustment		0.680	0.094	0.017	0.275	0.794	0.037

[a-c] Means within a column with different superscripts differ at $p < 0.05$

[1] Weight adjusted FCR was adjusted to an equal BW using 27g of body weight being equal to one point of FCR

[2] ECONASE XT, AB VISTA, Marlborough, UK. Xylanase was included in the diets at 16,000 BXU/kg at an inclusion rate of 100g/ton

[3] Non-adjusted diets were formulated using corn AME values from the NRC (1994). All corn sources were considered to have equal AME values, included at the same percentage, and formulated to be isonitrogenous and isocaloric

[4] Adjusted diets were formulated using the predicted corn AME values determined using an NIR and a calibration for corn AME developed by Aunir and AB Vista. The corns were included into the diets at different percentages and formulated to be isonitrogenous and isocaloric

[5] Means represent 20 birds per pen, 10 pens per diet

Table 6-9. Average body weight, cumulative mortality adjusted feed conversion ratio (cFCR), cumulative feed consumption (cFC), and cumulative waFCR of male broilers fed a diet with varying corn sources, with a NRC (1994) or predicted AME value of corn, and the addition of enzyme or control (2-way interaction between corn source and enzyme inclusion).

		Body Weight			**cFCR**	**cFC**	**waFCR**
Corn Source	**Xylanase**	**Day 18 (g)**	**Day 33 (kg)**	**Day 42 (kg)**	**Day 0-42**	**Day 0-42 (g)**	**Day 0-42**
A	Enzyme	640.4	2.064	2.981	1.608	110.9	1.580
A	Control	631.8	2.052	2.990	1.603	110.0	1.569
B	Enzyme	637.3	2.024	2.942	1.611	110.9	1.596
B	Control	621.2	2.024	2.917	1.621	110.5	1.614
C	Enzyme	625.4	2.028	2.950	1.633	110.0	1.615
C	Control	610.2	1.993	2.898	1.630	110.5	1.631
Main Effect Means							
Corn Source							
A		636.1[a]	2.058[a]	2.986[a]	1.605[b]	110.5	1.574[b]
B		629.2[a]	2.024[b]	2.929[b]	1.616[b]	110.7	1.605[a]
C		617.8[b]	2.011[b]	2.924[b]	1.632[a]	111.4	1.623[a]
	Xylanase						
-	Control	621.0[b]	2.023	2.935	1.618	110.3[b]	1.605
-	Enzyme	634.4[a]	2.038	2.958	1.618	111.4[a]	1.597
	p-value						
Corn Source		0.001	<0.001	<0.001	0.001	0.309	<0.001
Xylanase		0.001	0.079	0.064	0.941	0.045	0.332
Corn x Xylanase		0.678	0.266	0.118	0.483	0.574	0.276

[a, b] Means within a column with different superscripts differ at $p < 0.05$

[1] Weight adjusted FCR was adjusted to an equal BW using 27g of body weight being equal to one point of FCR

[2] ECONASE XT, AB VISTA, Marlborough, UK. Xylanase was included in the diets at 16,000 BXU/kg at an inclusion rate of 100g/ton

[3] Non-adjusted diets were formulated using corn AME values from the NRC (1994). All corn sources were considered to have equal AME values, included at the same percentage, and formulated to be isonitrogenous and isocaloric

[4] Adjusted diets were formulated using the predicted corn AME values determined using an NIR and a calibration for corn AME developed by Aunir and AB Vista. The corns were included into the diets at different percentages and formulated to be isonitrogenous and isocaloric

[5] Means represent 20 birds per pen, 10 pens per diet

Results

Body Weight. The main effect of corn source impacted d 18 BW of broilers with corn source A and B having a higher average male BW when compared to broilers fed corn source C (Table 6-7). Adjusting diets based on the AME prediction value resulted in an increase ($p<0.001$) in average male BW compared to using the NRC (1994) value for each corn source (Table 6-7). Significant interactions were present between corn source*adjustment factor on d 33 ($p<0.001$) and d 42 (Table 6-7). On d 33 a linear increase was observed in BW between corn sources when feeding diets using the NRC (1994) non-adjusted AME value for corn. Corn source A had the highest average BW compared to corn source C with corn source B being intermediate. However, when diet formulation was adjusted for the predicted AME of corn, performance was not statistically similar. On d 42 (Table 6-7) broilers fed diets with corn source A were heavier when compared to broilers fed corn sources B and C. Again, when diet formulation was adjusted for the predicted AME of corn using NIR and the prediction equation, performance was not statistically similar between corn sources. Xylanase inclusion had no impact on BW regardless of corn source or adjustment factor. There was a significant BW interaction ($p=0.017$) on d 42 between enzyme*adjustment factor (Table 6-8). Broilers fed diets adjusted for the predicted AME of corn had an increase in BW when compared to broilers fed non-adjusted diets. When including the enzyme into the non-adjusted diets, BW was increased, however not to levels similar to the adjusted diets.

Cumulative Mortality Adjusted Feed Conversion Ratio. An interaction was present between corn source*adjustment factor for d 0 to 42 regarding cumulative FCR (Table 6-7). No differences were observed for corn source A regardless of adjustment factor, however, broilers fed diets adjusted for predicted AME value on corn source B and C resulted in a reduction ($p=0.001$) in cumulative FCR compared to the non-adjusted value through d 0 to 42. Differences were not observed between corn sources when diets were adjusted for the predicted AME of corn. Xylanase inclusion had no effect on cFCR regardless of corn source or adjustment factor (Table 6-8).

Cumulative Body Weight Adjusted Feed Conversion Ratio (waFCR). An interaction was present between corn source*adjustment factor for d 0 to 42 regarding cumulative waFCR (Table 6-7). Observations for corn source A resulted in no differences regardless of adjustment factor, however, broilers fed diets adjusted for predicted AME value on corn source B and C had a reduction ($p<0.001$) in cumulative waFCR compared to the non-adjusted diet for d 0 to 42 at 9 points and 10.6 points for B and C corn sources, respectively. There was also an interaction between enzyme*adjustment factor (Table 6-8). Broilers fed the adjusted diets had a decrease in waFCR when compared to non-adjusted fed broilers. When including the enzyme into the non-adjusted diets, waFCR was decreased, however not to levels similar to the adjusted diets.

Cumulative Feed Consumption. Enzyme inclusion increased cFC for d 0 to 42 by 1.1 g/bird/day regardless of corn source or adjustment factor (Table 6-8).

Discussion

Corn is a main contributor of ME in broiler starter diets at approximately 65% and also contributes about 20% protein (Cowieson, 2005). Variation in corn nutrients can potentially damage broiler performance and increase production costs (Socorro et al., 1989; Leigh, 1994; Cowieson, 2005; Gehring et al., 2012; Gayral et al., 2015). There are numerous factors that can influence corn nutrient variability and digestibility. Thus, the ability to accurately predict the AME of corn could be a valuable tool in diet formulation. If AME of corn could be rapidly and accurately predicted, it may result in the possible prediction of xylanase response, leading to more efficient nutrient utilization and maximizing broiler performance. Although predicting ingredient nutrient values and exogenous enzyme response is a challenge, the current study uses a calibration that showed to be advantageous when predicting the AME of corn (Masey O'Neill et al., 2013, 2014). Using the prediction equation reflects more accurate nutrient content prior to diet formulation allowing for more efficient diet formulation. Corn samples in the current trial were determined to have a 105 kcal/kg difference in AME from the highest quality corn to the lowest (Table 6-1). Protein content of the corn samples also differed with about 0.4% difference between corn sources. Differences in these analyzed corn nutrient values combined with the differences in observed growth performance are evidence that not all sources of corn should be formulated in poultry diets based on generic nutrient content values.

During the starter phase, FC was increased by 0.5 g/bird/day with the inclusion of xylanase. Adjusting the diets for predicted AME of corn resulted in almost a 1

g/bird/day increase in FC during the starter phase. Enzyme inclusion increased cFC for d 0 to 42 by 1.1 g/bird/day regardless of corn source or adjustment factor. Collins and Moran (2001) reported dissimilar results observing significant differences in FC with regards to corn source; however, differences could be due to differing kernel characteristics, which was not evaluated in the current experiment. Once diet formulation was adjusted for the predicted AME values, no differences between corn sources were observed. This supports the theory that using accurate prediction models for corn AME values can equalize broiler performance in the presence of nutrient variation. When feeding enzymes, FC can be variable. This is due in part to the hydrolyzation of polysaccharides involved in encapsulation of starch and protein, making nutrients readily available to the bird (Bedford, 1996). The addition of xylanase to the diets increased FC in all treatments cumulatively. Inconsistent results with enzyme inclusion in corn-SBM diets have been well documented (Bedford and Classen, 1993; Kocher et al., 2003; Cowieson et al., 2006; Yagani and Korver, 2013). Yegani and Korver (2013) reported mixed results with some enzymes included increasing FC while other enzymes had no effect. According to Cowieson (2010), the nutrient quality of the diet is perhaps the most important factor that impacts response to an enzyme. In the current experiment, increases in cFC were observed with both the addition of xylanase to diets and with adjusting diets for the predicted AME of corn.

Corn source, xylanase inclusion, and adjustment factor all impacted ($p \leq 0.001$) d 18 BW independently. Observations in corn source resulted in differences in over 18 grams of BW for d 18. Formulating diets using the NRC (1994) AME values for all

corn samples, broilers fed corn source A had the highest average BW with a 2.9% increase over corn source C. Adjusting diets based on the AME prediction increased d 18 BW by 29.2 g compared to the non-adjusted diets. Similarly, the inclusion of xylanase increased d 18 BW by 13.4 g compared to the control. As such, additive improvements in early BW were achieved through xylanase inclusion and adjusting diets based on AME prediction. Following d 18, interactions were observed corn source*adjustment factor. No differences were observed on d 33 and d 42 BW in corn source A with xylanase inclusion or adjusting diets based on AME prediction as this was the corn source with the highest and closest value to NRC and would not expect dramatic impacts associated with reformulation based on the predicted value. However, broilers fed diets adjusted for predicted AME value increased d 33 and d 42 BW in corn source B and C compared to the non-adjusted diets. These observations validate that the differences in BW are due to the varying nutrient profiles of the selected corn sources and reformulation with accurate AME values eliminates any growth differences associated with nutrient variability. These results support that using an accurate AME prediction equation when formulating diets balances broiler performance; even with facing severe variations in corn quality. In a study conducted by Collins and Moran (2001), similar results were observed with corn source impacting average BW. The addition of xylanase to the adjusted diets did not have a significant effect on BW whereas it was effective in increasing the BW of those birds fed the 2 lower energy corn sources when the diet was not adjusted for these differences. Previous publications support these results in which enzyme inclusion has shown to be advantageous to

improving broiler performance in reduced energy diets (Bedford and Classen, 1993; Wang et al., 2005; Esmaeilipour et al., 2011; Coppedge et al., 2012; Masey O'Neill et al., 2012; Williams et al., 2014).

Starter phase FCR was impacted by corn source with about a 2 point increase in corn source C compared to the other corn sources. Adjusting the diet for the predicted AME of corn resulted in a 4 point reduction if FCR for the starter phase. These data support that variety in corn nutrient profiles can significantly impact feed conversion ratio, however, formulating with accurate AME values will alleviate flock performance variation. Inaccurate AME values (using an equal value for all corn sources) is extremely costly based on growth performance data. Due to the 86 kcal/kg difference in corn AME, the non-adjusted diets had differences in calculated AME of 58 kcal/kg between corn A and corn B resulting in a 4 point reduction in cFCR through d 42. Non-adjusted diet differences were observed in the cFCR through d 42 with corn source A having the lowest FCR which was predicted to have the highest AME value. The 105 kcal/kg difference in AME between corn A and corn C resulted in a difference of 70 kcal/kg in AME for the non-adjusted diets. The difference in the non-adjusted diets resulted in a 6 point reduction in the cFCR through d 42. Once diet formulation was adjusted with predicted AME values, cFCR was not different between corn sources. However, adjusting the diet formulation for predicted AME did result in a significant reduction in cFCR in the B and C corn sources when compared to the non-adjusted diets. As differences were observed in d 42 BW, FCR was adjusted to an equal BW using 27g of body weight being equal to 1 point of FCR. A significant interaction occurred

between corn source and adjustment factor for the waFCR. When diets were adjusted for the predicted AME, there was a 9 and 10 point reduction in waFCR for corn source B and C, respectively, when compared to the non-adjusted diet. These data demonstrate the negative impact on broiler performance that can occur due to variability in corn sources. Corn source A resulted in only a 2 point reduction in waFCR, versus a 7 to 10 point reduction in corn source B and C. This is because the nutrient value of corn source A was the most closely related to the NRC (1994) corn values. Throughout the study, as the predicted AME value of corn was closer to the NRC (1994) value, differences became less pronounced. These data support the concept that using a non-adjusted value for ingredients will not allow for accurate diet formulation because of the variation in composition and variety. Accurately predicting corn AME values allows for more effective diet formulation. Adjusting diet formulation for the predicted AME of corn leads to a more balanced diet, improving broiler performance and flock uniformity. In the absence of adjustment, the addition of the xylanase lead to a significant improvement in waFCR, driven mostly by weight gain effects, whereas in the presence of adjustment this effect was removed. Enzyme use is known to reduce variability in performance between cereal sources, and this was clearly demonstrated in this experiment . Commercially, few adjustments are made to the formulation with deliveries of new corn samples, and as a result the performance would vary as much as the corn samples varied, similar to the non-adjusted data in this study. The maximum benefit would be achieved if attention was given to the incoming corn quality and diets re-formulated accordingly. However, in the absence of such a reactive response, the inclusion of the enzyme

mitigates some of the differences noted between the corn samples during the starter phase by delivering the most benefit on the non-adjusted corn sources.

CHAPTER VII

CONCLUSION

Reducing feed costs while improving broiler performance and nutrient utilization are primary areas of focus for poultry nutritionists. Several methodologies are currently available to nutritionists to aid in achieving these goals. The methods utilized in the current experiments are commonly used in the poultry industry, and can help to provide more relevant data when formulating feeds for the modern broiler.

When investigating the effect of β-mannanase sourced from *Bacillus subtilis* on broiler performance, ileal digestible energy, and intestinal viscosity (Experiment 1), the PC fed broilers maintained a heavier average BW ($p<0.05$) when compared to the NC between d 14 and d 42, indicating that the reduction in energy was sufficient to reduce average BW. With the inclusion of β-mannanase in the NC, broilers were able to achieve similar performance when compared to the PC during this period. The PC diet maintained a lower mortality adjusted FCR ($p<0.05$) when compared to the NC during weeks 1 and 2 while the inclusion of β-mannanase produced similar results as the PC. The PC fed broilers exhibited an improved cumulative FCR ($p<0.05$) compared to the NC diet throughout the duration of the experiment while the β-mannanase inclusion resulted in a similar FCR as compared to the PC fed broilers. Ileal digestible energy was increased by 55 kcal/kg with the inclusion of β-mannanase as compared to the negative control diet on d 17 of age; however, this increase was not observed on d 37. Although there was no significant effect of β-mannanase on intestinal viscosity or IDE, growth improvements in broilers fed a reduced energy diet in Experiment 4 may be

related to a reduction in the feed induced immune response associated with the galactomannan presence in SBM. The improvements in growth performance associated with inclusion of β-mannanase were more pronounced early during grow-out which corresponds with higher levels of soybean meal in the starter diet as compared to the withdrawal diet. Overall, β-mannanase inclusion eliminated losses in growth performance associated with reductions in dietary energy.

When investigating the impact of β-mannanase inclusion on growth performance, viscosity, and energy utilization in broilers fed diets varying in GM concentrations (Experiment 2), increasing levels of GM negatively ($p<0.05$) influenced BW following the starter and grower periods and increased mortality corrected FCR throughout the study. Reduced growth performance was associated with increased ($p<0.05$) intestinal viscosity and decreased ($p<0.05$) IDE when GM inclusion was increased. Inclusion of β-mannanase in diets containing supplemental GM improved average male BW to levels similar to diets without supplemental GM. Improvements in FCR were also observed with β-mannanase inclusion in diets containing supplemental GM. Ileal digestible energy was increased with β-mannanase inclusion ($p<0.05$). Multiple interactions in growth performance, intestinal viscosity, and IDE were associated with β-mannanase administration. Inclusion of dietary GM had a negative impact on broiler performance throughout the trial with the highest concentration consistently having the largest impact. However, inclusion of β-mannanase was able to mitigate negative effects of GM content in the diet, particularly during the grower phase of the trial. Intestinal viscosity was increased ($p<0.05$) as GM concentration increased in the diet. Beta-mannanase inclusion

reduced intestinal viscosity, however, not to levels similar to control. Ileal digestible energy was significantly increased as dietary β-mannanase inclusion increased. However, performance improvements were dependent upon GM concentration.

When determining the dSAA of male Cobb 500 broilers from 35 to 49 d (Experiment 3), all parameters evaluated showed strong relationships for dSAA level, except for FC. The dSAA requirements that maximize d 49 BW, BWG and minimize FCR and cFCR were 0.813, 0.823, 0.772, and 0.779%, respectively. Broiler performance responded in a curvilinear relationship to DLM with the exception of feed consumption. Broilers did not respond to higher incremental levels of DLM with the maximum benefits at the 84:90 dSAA:dLys ratio. The dSAA requirement for BWG was 0.823%, with the requirement being lower for FCR at 0.772%. No differences were observed between the 2 Met sources confirming that the bioavailability value of HMTBa is 100%.

When investigating the effect of corn AME value, on broiler performance associated with geographical location (source), xylanase inclusion, and formulation (Experiment 4), xylanase increased ($p<0.05$) starter feed consumption and d 18 BW (2.1%), and an increase in BW (4.6%) was observed using the predicted AME values. Corn source continued to impact broiler performance throughout the remainder of the experiment as corn source A consistently outperformed ($p<0.05$) corn source C with increased BW and reduced mortality corrected FCR. Dietary adjustment with AME predicted values for each corn source consistently improved ($p<0.05$) growth performance. Corn source A had the highest average BW with a 2.9% increase ($p<0.05$)

over corn source C. Early FCR was improved ($p<0.05$) with the predicted AME value diet fed broilers, decreasing FCR by 3%. Multiple significant interactions were observed between the corn source and the adjustment method. For example, with d 42 FCR, using the predicted AME value adjustment had an impact of 9 and 10 points for corn source B and C, respectively, but only 2 for corn source A. Variations in corn nutrient content can be detrimental to broiler growth performance if a static AME value is used for formulations. Xylanase inclusion increased early BW and had the greatest impact on the non-adjusted corn diets with the lowest AME values. The nutrient value variation of corn samples with the US can result in significant performance reductions as a 12 point difference in waFCR was observed between the corn sources used. Accurate prediction of AME value can alleviate growth performance variability associated with drifts and differences in corn nutrient content. Reformulation with accurate values in the current study resulted in improvements of 11 points of waFCR. This experiment demonstrates the impact of variable corn nutrient content and the potential improvement when using NIR technology for ingredient nutrient specifications.

Ultimately, these data indicate the ability of exogenous enzyme inclusion, methionine requirement, and ingredient variability to impact broiler performance and nutrient digestibility. Inclusion of exogenous enzymes such as β-mannanase and xylanase can improve broiler performance and nutrient digestibility. Methionine requirement for the modern broiler in later phases of growth may be higher than what previous research suggests. Furthermore, ingredients should not be considered equal when formulating diets.

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